NUTRIENT UPTAKE AND EXUDATION PATTERNS OF NITROGENOUS SUBSTANCES AND POLYPHENOLS IN THE KELP *Ecklonia maxima* (Osbeck) Papenfuss.

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ABSTRACT

Exudation patterns of organically bound nitrogen and polyphenol substances, as well as the uptake of nitrate, ammonium and phosphate were studied in the kelp Ecklonia maxima (Phaeophyceae) in ex-situ experiments. It was found that exudation of organic materials only rarely followed a straight accumulatory trend ; fluctuations of kelp-derived substances occurred as a net result of pulsating production patterns coupled with differential rates of utilization by marine heterotrophic organisms. Pulsing took place at irregular intervals in different plants, precluding statistical treatment of exudation. The observed initial pulsing liberation of DON and polyphenols, affected the quantification of exudation rates which could only be tentatively determined.

Antibiotic agents used in one experiment did not succeed in controlling free-living bacteria in the water column without affecting the kelp : DOM production was diminished in the presence of antibiotics, leading to the conclusion that they affected the physiology of the epidermal cells. This provides indirect evidence that exudation is an energy-dependent, active process.

Measurements of uptake velocities of ambient and enriched nitrogen established that there was a higher affinity for ammonium as a nitrogen source than for nitrate at enriched N levels. At ambient nutrient levels, nitrate uptake velocities were 1,5 times higher than those for ammonium, making nitrate the primary nitrogen resource of E. maxima. However, the uptake potential for either source was not competitively inhibited by the other. Ammonium and nitrate uptake velocities still increased from enriched levels of 30-40 uM N to approximately 100 uM N. Both exudation and uptake phenomena were subject to seasonality : During summer, the rates for exudation and uptake were found to be higher than in winter, which is known to be a dormant phase in the growth of E. maxima.

A comparative analysis of dissolved free amino acids in the water surrounding the kelp and a tissue analysis of free amino acids obtained under enriched conditions showed that alanine was prominent both in the thallus and in the water. Glutamic acid was detected in the water only after the kelp had been introduced during a nitrogen feeding experiment and appeared to be GS/GOGAT derived. Aspartine was also detected at high relative concentrations.

Polyphenolic substances (phlorotannins) accumulated in exudates of E. maxima more readily due to the low biodegradability of these compounds and contributed to the yellow colouration and UV-absorption characteristics (Gelbstoff) of the surrounding water.

UITTREKSEL

Die seewier Ecklonia maxima (Phaeophyceae) is 'n hoofprodusent van biomassa langs die suidwestelike kus van Suidelike Afrika. Die opname van nitraat, ammonium en fosfaat is ondersoek in verband met die uitskeidingspatrone (= exudation patterns) van organies gebonde stikstof en polifenole gedurende ex-situ eksperimente. Dit het getoon dat die uitskeiding van organiese stowwe slegs in enkele gevalle 'n reguit toenemende patroon wys ; fluktuasies van kelp-afgeleide stowwe word sigbaar as 'n gevolg van pulserende produksie patrone verbonde met onderskeidelike snelhede van benutting deur marienheterotrofiese organisme. Polse het in onreëlmatige afstande voorgekom, wat die statistiese behandeling van uitskeiding bemoeilik het.

Antibiotika wat in een eksperiment gebruik is het nie geslaag om bakterie in die water te beheer sonder om die seewier aanteraak nie : Die produksie van opgeloste organiese stowwe het in aanwesigheid van die antibiotika afgeneem, wat aanleiding gee tot die gevolgtrekking dat hulle die epidermiese selle van die plant aantas. Dit word as 'n afgeleide bewys aangevoer dat uitskeiding 'n energie-afhangende en aktiewe proses verteenwordig.

Die bepaling van opname-snelhede van omringende en verrykde konsentrasies van stikstof het 'n hoër aantrekkingskrag vir ammonium in plaas van nitraat as 'n stikstof-bron aangetoon wat verrykte vlakke van stikstof aanbetref. Op omringende voedingsvlakke was nitraat opname-snelhede 1,5 keer hoër as die vir ammonium wat nitraat die primêre stikstofbron van E. maxima maak. Die opname-vermoë was egter nie beïnvloed of mededingend onderdruk deur die teenwoordigheid van die ander stikstofbron nie. Ammonium en nitraat opname-snelhede het bokant 30-40 uM N nog steeds toegeneem tot vlakke van 100 uM N. Albei prosesse, uitskeiding en opname, word deur die jaargetye beinvloed : Daar is hoër snelhede van uitskeiding en opname gedurende die somer gevind vergeleke met die winter, wat algemeen bekend staan as 'n rusperiode van E. maxima - groei.

'n Vergelykende studie van opgeloste ongebonde aminosure in die omringende water en 'n weefsel analise van die kelp wat onder verrykde toestande verkry is, het aangetoon dat alanien teenwoordig was in thallus en seewater. Glutamiese suur was in die water te bespeur eers nadat seewier bygevoeg is gedurende 'n stikstof-bemestings exksperiment, en dit het voorgekom asof die suur afkomstig was van die GS/GOGAT sisteem. Daar is ook relatief hoë vlakke van aspartien gevind.

Polifenole (florotannine) het meer geredelik in uitskeidings van E. maxima weens die moeilike afsetting van hierdie verbindinge akkumuleer en het bygedra tot die geel kleur en UV-absorbeerende eienskappe van daardie uitskeidings (Gelbstoff) in die omringende water.

I N D E X

CHAPTER 1

General Introduction

CHAPTER 2

Materials and Methods

CHAPTER 3

Exudation of Nitrogenous Substances by Ecklonia maxima and their Heterotrophic Utilization

CHAPTER 4

Nutrient Uptake and Assimilation Studies on E. maxima

CHAPTER 5

Exudation of Polyphenols and UV-Absorbance of Exudates

CHAPTER 6

CHAPTER 7

C H A P T E R

I

GENERAL INTRODUCTION

1.1. THE KELP ECOSPHERE

The rocky subtidal habitats in most coastal, oceanic areas of temperate regions are conspicuously dominated by large brown algae (Phaeophyta). Those phaeophytes belonging to the order Laminariales are commonly referred to as kelps. Kelps reflect a high degree of biological organisation and their high relative biomass contribution allow them to occupy a dominant position in the dynamics of a typical kelp-bed ecosystem (Dayton, 1985).
Kelp forests have conventionally been classified by the presence of their prominent genera (see Mann, 1982). Laminaria species are present on both sides of the Atlantic Ocean, with large populations along the eastern Pacific coast. The giant kelp, Macrocystis, forms dense forests in many parts of the world, for example, the southern shores of South America and many Southern ocean islands. The Macrocystis kelp-beds growing off the Californian and Oregon coasts are well-known and have been studied in detail as well as commercially exploited (Lüning, 1985; Lobban, et al, 1985).

Along the South African coast, Ecklonia and Laminaria kelp communities are found in the inshore region of the coast directly or indirectly affected by the cold, northward flowing Benguela current originating in Antarctic waters. The Benguela current provides sporadic bursts of nutrient-rich waters to the south-western and western coasts of Southern Africa during upwelling cycles. These are influenced by wind direction and intensity, providing primary producers with upwelled nitrate and phosphate (Chapman & Shannon, 1985).
Together with phytoplankton organisms, the kelp beds extending from Lüderitz/Namibia to Cape Agulhas at the southernmost tip of Africa along the south-western coast constitute the principal

primary producers of the inshore region (Jarman & Carter, 1981). Productivity, as determined by measuring frond elongation rates, appears to be most strongly influenced by light rather than other environmental factors in South African kelps. Biomass production is lowest in winter and highest in early summer, corresponding roughly to the annual seasonal cycle (Dieckmann, 1980). Because of their small size and short generation time, planktonic organisms are influenced markedly by nutrients with growth closely linked to upwelling, whereas ~~seaweeds~~ Laminariales are less immediately affected by those short bursts of upwelled nutrients. (Bolton & Levitt, in press)

Ecklonia maxima (Osbeck) Papenfuss is usually found in shallow water (ca. 0 - 8 m depth range), whereas Laminaria pallida (Grev) J.Ag. is dominant at greater depths up to 20 m (Jarman & Carter, 1981). E. maxima has a hollow gas-filled stipe with a buoyant swollen bulb at the top, allowing its fronds to float at or near the surface. This is significant in terms of the ecology of kelp-beds as follows :

Firstly, E. maxima harvests light efficiently shading understorey algae, and secondly, if plants are torn free they are often cast ashore providing a nutrient input into sandy beach ecosystems (Branch & Branch, 1981).

In terms of biomass, Ecklonia maxima is clearly the dominant form - it accounts for up to 65-70 % of the total benthic biomass of the inshore region (Jarman & Carter, 1981). E. maxima therefore plays a leading role in the maintenance and energy-flow of its environment (Field, et al, 1980).

Kelps interact with the ambient seawater in two ways : The anabolic level resulting in uptake of nutrients and inorganic carbon from the sea leading to assimilation and production of metabolites. On a catabolic level, substances are released into

the water, cells and tissues degrade and form the basis of decomposition processes in the sea. Both processes are interlinked and can be separated for research purposes only with difficulty. Uptake and exudation phenomena are thus integral components of the nutrient physiology and energy-flow in Ecklonia maxima.

1.2. DISSOLVED ORGANIC MATTER (DOM) IN SEAWATER

Organic matter in seawater occurs in two forms : dissolved and particulate organic matter (DOM and POM). Conventionally, a filter size of 0.45µm has been adopted as a cut-off point between DOM and POM (Wangersky, 1978).

DOM and POM can be further classified into DOC, DON, DOP, and also POC, PON, POP (C, N, P representing carbon, nitrogen and phosphorus, respectively). DOC includes carbohydrates, polyphenols, sugars, polysaccharides and fatty acids, but excludes proteins, polypeptides and amino acids which fall under the collective term of DON (Wangersky & Zika, 1978). Biochemical metabolites such as adenosine triphosphate (ATP) form part of DOP.

However, organically bound N and P are labile and easily degradable in the marine environment, and are present as part of the total DOM and POM pool to only a small degree. The majority of N and P is present in an inorganic, ionic form.

Therefore most of the DOM is DOC. DOM is generally present at background levels of about 1-100 µg dm^{-3} in seawater, depending on whether a sample has been taken at an offshore or inshore site ; the former characteristically containing less DOM per unit volume than the latter. Depth also seems to affect the distribution of DOM. At greater depths less DOM is encountered,

primarily because of a lack of input from extracellular planktonic or benthic matter (Thomas, 1971).

DOM in seawater can originate via two pathways (Fig. 1):
A terrestrial source can contribute DOM to the sea via rivers and run-off. These substances typically include plant material, animal wastes or matter anthropogenic in origin. Alternatively, the DOM can be of an autochthonous origin, indicating a marine genesis (Valiela, 1984).
In the latter case, the material stems from excretory processes of consumers or more directly, from breakage or lysis of primary producers, eg. phytoplankton. Loss of DOM from cells damaged during grazing may amount to 15-20% of the consumed carbon - part of a phenomenon often termed "sloppy feeding" (Lampert, 1970).
Because of the fact that most of the organically bound carbon undergoes heterotrophic utilization and is reduced to carbon dioxide (CO_2), the largest single source of DOC in aerobic situations may be due to extracellular release by phytoplankton cells and macroalgae (Valiela, 1984).

Estimates of phytoplankton exudation vary widely: from 7 to 62% of photosynthetically fixed carbon (Hellebust, 1967 ; Choi, 1972; Larsson & Hagström, 1979). Exudation rates in phytoplankton are strongly affected by coastal upwelling and nutrient availability; it is generally accepted that <u>in situ</u> rates of release are clustered more towards the lower end of the spectrum and are currently estimated to be around 10 to 20 % (Valiela, 1984).

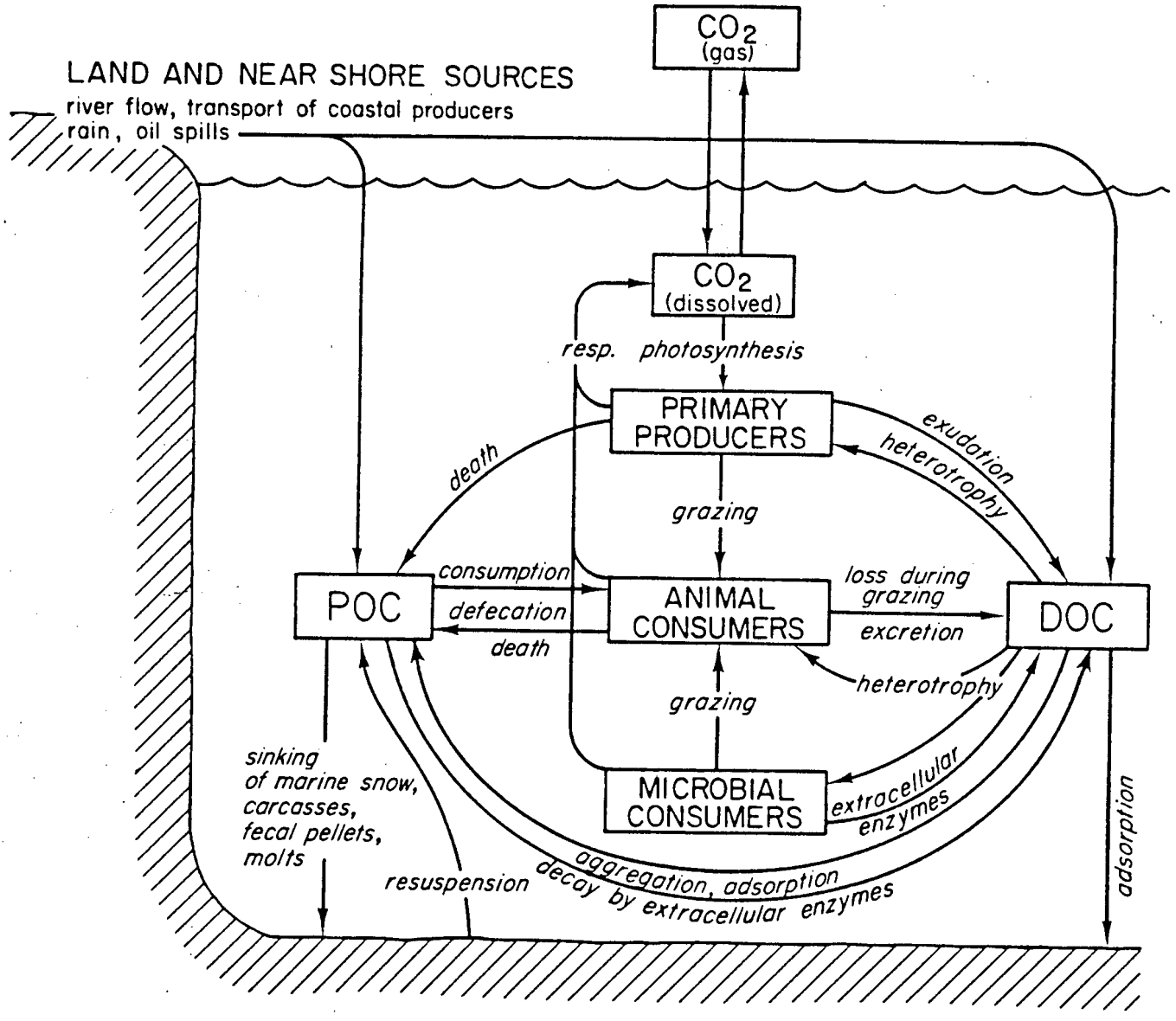


Fig. 1 : The flow of Organic Matter in the Marine Environment. (from : Marine Ecological Processes, Valiela, 1984)

1.3. EXUDATION PROCESSES IN SEAWEEDS

Unicellular algae and seaweeds are known to absorb nutrients for vital structural and metabolic processes, and have also been found to release minor quantities of organic compounds during a process called extracellular release or exudation (Fogg & Boalch, 1958).

Apart from obvious physically degradative processes such as the erosion of fronds and sloughing-off of surface cells resulting in the production of POM, the material released during exudation is DOM (refer to Fig. 2).

The material exuded consists to a large extent of DOC : These are carbohydrates, sugars and alginates, as well as polyphenols (Sieburth & Jensen, 1969 ; Newell, et al, 1980). These substances together with the N-containing compounds (proteinaceous material, urea, peptides, amino acids) are degraded by marine heterotrophs (bacteria) at a rapid rate (Hobbie, et al, 1972). In particular, mucilage (polymucosaccharides) that is released from kelp fronds upon injury is vigorously attacked by bacteria (Newell & Lucas, 1981). Following this, an ecological succession develops, with flagellates and ciliates feeding on the bacteria (Linley & Newell, 1981). Once the nitrogen requirements of the consumers are fulfilled, N is liberated and enters the system again as dissolved inorganic nitrogen (DIN). Not all the DOC and DON is completely utilized. Refractory residual matter that is resistant to bacterial attack persists in the marine environment and forms part of "Gelbstoff" substances, so called, because of their yellow and UV-fluorescing properties (Kalle, 1966). Most of the Gelbstoff, however, is derived from a particularly important fraction of the DOC-pool ; namely, the polyphenols.

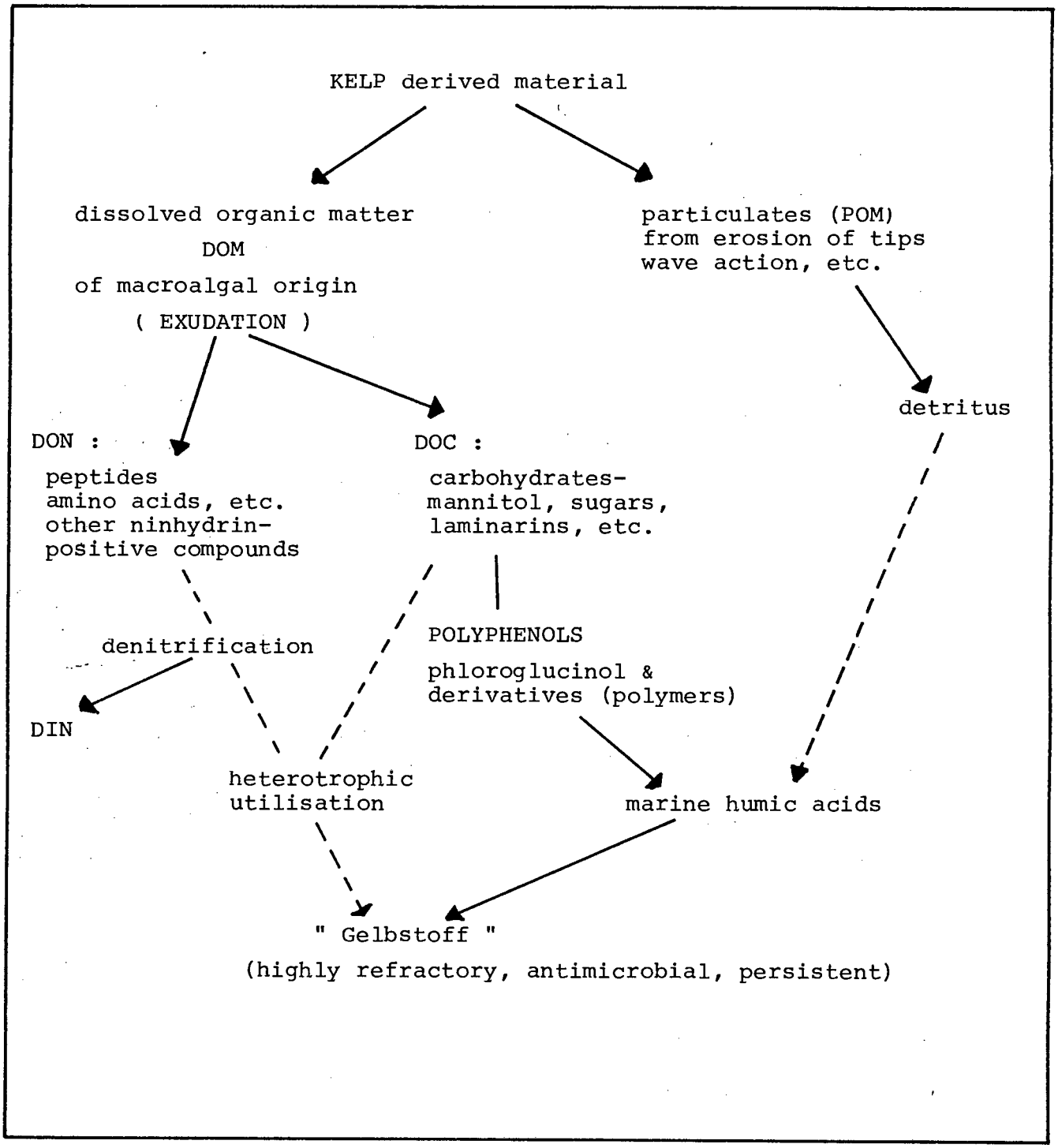


Fig. 2 : The Degradative Pathways of Kelp-Derived Material.

Polyphenols undergo incomplete biological transformation and, coupled with purely chemical reactions such as oxidations and polymerisation, this results in the formation of marine humic acids (Duursma, 1965). Together with related marine fulvic acids, these show a pronounced affinity to the analogous terrestial substances (Skopintsev, 1981). Similarly, marine humic acids are resistant to rapid bacterial decay and thus form the major part of the Gelbstoff pool. It has been postulated that Gelbstoff can also form as a result of marine humic acids combining with the residual DON material and exuded proteinaceous matter (Sieburth & Jensen, 1969). The POM produced undergoes bacterial colonisation and is successively utilized by filter-feeders, egested and recolonised, enriching the particles with bacterial nitrogen in the process which ultimately benefits the kelp bed community (Newell, et al, 1980).

The function of exudation is not clear and no single satisfactory hypothesis of the purpose of exudation has been offered to date. Extracellular release could be interpreted as an excretory process : The seaweed, possessing primitive cell walls and only limited storage capabilities from an evolutionary point of view, is able to rid itself of excess photosynthetic metabolites by simple leaching.

Evidence for this is that exudation takes place mainly during the day, leading to the conclusion that exudation is somehow linked to photosynthesis and the current carbon storage-state of the algal macrophyte (Sieburth & Jensen, 1969). More recently, Carlson & Carlson (1984) have found extracellular release to be low in winter, under overcast conditions and at night, supporting the earlier findings. Similarly, Brylinsky (1977) reported an approximately 10-fold reduction in the liberation of DOC by some marine macroalgae.

It has been suggested that DOM of algal origin can complex metals because of its metal chelating properties (Ragan & Jensen, 1980). Because solubility constants of Fe^{2+} and Fe^{3+} as well as manganese (Mn^{2+}) are lower in seawater than in freshwater, these ions usually precipitate. Iron and manganese are therefore scarce and highly limiting in the growth of marine autotrophic organisms. Measuring the metal complexing ability of exudate after a 30-day culture period of selected red and brown seaweeds, Sueur, et al, (1982) determined the concentration of metal complexing ligands released into the media. The metal complexing ability of the solution was found to increase with increasing concentrations of exudate.

Algally derived DOM can influence other organisms by its toxicity as repeatedly demonstrated (eg. toxicity of exudate to fish larvae, Sieburth & Jensen, 1969). Further, the anti-fouling properties of algal extracts are documented (Sieburth & Tootle, 1981).

Geiselman and McConnell (1981) found that the polyphenol fraction of brown algae present intracellularly as well as in extracellular DOM, reduces or completely inhibits food intake in the marine herbivorous snail Littorina. They concluded that these secondary metabolites were not functionally different from terrestrial plant polyphenols (tannins) which play an important role in the chemical defence against herbivores. Algal DOM appears to have a regulatory function, maintaining low herbivore levels in kelp-dominated ecosystems to ensure steady recruitment of young kelp. However, the quantities of phenolics necessary to influence the number of herbivores would have to be very high to compensate for the rapid turnover in an open system.

As a feeding deterrent, exuded algal DOM is therefore most

probably of less importance. The antibacterial properties of some of the substances present in exudate solutions - notably the polyphenol fraction - are known and are thought to function as antibiotics or allelopathic chemicals to reduce or eliminate pathogens and competitors (Vadas, 1979).

Extracellular release of dissolved organic material affects the chemistry and therefore the ecology of inshore waters, depending on the quantities of algal macrophytes present in the system (Barnes & Mann, 1982). There are no analogous processes of metabolite loss in higher terrestrial plants with the exception of volatile compounds (scent) and floral nectar, both of which are known to fulfil a definite ecological or evolutionary function (Vadas, 1979).

The processes noted above appear insufficient to justify the expenditure of photosynthate gained at cost by the plant.

It could be postulated that exudation is a laboratory artifact - a release of substance prompted by a shock response on the part of the plant. Moebus and Johnson (1974) have pointed out that harvesting or handling of the algae may have influenced release. It is evident from the literature that there is a trend away from disruptive techniques and towards simulated *in situ* studies to minimise on these potential interferences. Additionally, exudate collection and accumulation is studied over a course of 1 to 2 or maximally 3 hours (Carlson & Carlson, 1984) while earlier studies have measured exudation in time scales ranging from 4 to 5 hours (Khailov & Burlakova, 1969) over 10 to 14 hrs (Brylinsky, 1977) to long-term studies of 20 to 24 hrs (Sieburth & Jensen, 1969). A considerable percentage of DOM release values thus derived may also incorporate leaching of metabolites from damaged cells, cell lysis and mucilage production in response to wounding, and also bacterial POM/DOM conversion processes (Linley *et al*, 1981).

1.4. NUTRIENT UPTAKE IN SEAWEEDS WITH PARTICULAR REFERENCE TO NITROGEN

Seaweeds differ from higher plants in that they are able to absorb nutrients from the surrounding seawater through their whole thallus. Moreover, most are able to accumulate certain elements in their thalli, such as iodine for example, which are present in seawater only in trace amounts (Floc'h, 1982).

The normal biochemistry of plants requires a number of specific elements to be present. There is overwhelming evidence that C, H, O, P, N, Mg, Fe, Cu, Mn, Zn, Mo, S, K, and Ca are required by all algae, although S, K, and Ca may be replaced to some extent by other elements in certain algae (deBoer, 1981).
Comparing the elemental composition of seaweeds with that of average seawater, a number of elements are seen to be severely limiting. There are indications that N, P, Fe, and possibly Mn and Zn are generally in shortest supply and may limit the growth of seaweeds, since their enrichment factors are all of the order of 10^5 (after deBoer, 1981).

Elemental nitrogen is abundant in air and dissolved in seawater. Until recently, it was thought that only a few cyanophytes were able to fix N_2 in the sea and hence utilize it directly. However, evidence is mounting that unicellular green algae are also able to perform nitrogen fixation, eg. in a strain of Chlorella (Weathers, et al, 1984).
The main inorganic forms of nitrogen in all major ecosystems are: Nitrate (NO_3^-), ammonium (NH_4^+) and to a minor extent, nitrite (NO_2^-) (Barnes & Mann, 1982).
Most algae, including seaweeds, use ammonium preferentially; that is, when an algae is supplied with both NO_3^- and NH_4^+, nitrate is often not being utilized until most of the ammonium ions have

been used up (deBoer, 1981). However, this does not mean that nitrate is less effective in promoting growth ; Codium fragile subsp. tomentosoides (Goor) Silva was found to grow just as well irrespective of whether NO_3^-, NO_2^- or NH_4^+ was used as a nitrogen source (Hanisak, 1979). Similarly, Chondrus crispus Stackh. has been shown not to demonstrate any marked preference for either N source (Neish & Shacklock, 1971).

DeBoer, et al (1978) determined that inorganic nitrogen is generally preferred by seaweeds over organically bound nitrogen (such as amides or urea). Again, this does not seem universally applicable. Ulva fasciata Raff-Del. exhibited higher growth rates when supplied with urea [$(NH_2)_2CO$] than either type of inorganic N (Mohsen, et al, 1974). Seaweeds have been reported to possess the ability to store nitrogen for use when external nitrogen levels are at a minimum. Along the coast of Nova Scotia, Laminaria longicruris Pyl. has been found to concentrate NO_3^- as much as 28 000 fold during periods of N sufficiency. During the summer months, the seawater is almost completely devoid of N, but owing to the large internal reserves of N, growth can still take place (Chapman & Craigie, 1977).

Nitrogen has to be stored as nitrate, since ammonium is toxic to plants at higher concentrations and has to be assimilated into various carbon skeletons immediately, thus placing a strain on the carbon reserves of the macroalga (Bidwell, 1979).

Ion fluxes across membranes may result from passive diffusion, facilitated diffusion, exchange diffusion or active diffusion. There is also the phenomenon of uptake into the so-called "apparent free space" (AFS), which denotes a rapid, quasi soaking-up of ions into the intercellular spaces. AFS designates the volume accessible by diffusion and ion-exchange components external to the plasmalemma (Bidwell,1979).

A more comprehensive introduction to nutrient uptake measurements and nitrogen assimilation will be given in chapter 4.

1.5. AIMS AND OBJECTIVES OF THIS STUDY

Exudation of organic substances and uptake of inorganic nutrients was studied in the southern hemisphere kelp Ecklonia maxima (Osbeck) Papenfuss [Alariaceae, Laminariales, Phaeophyceae]. The preceeding literature review has identified certain objectives as follows :

i) Measurement of exudation by monitoring the appearance of organically bound nitrogen and polyphenols in water surrounding kelp in a closed experimental system.

ii) Determining uptake of nitrogen and phosphorus by the disappearance of these compounds from ambient seawater.

iii) Assimilation of added nitrogen into the thallus.

iv) The influence of kelp on amino acid composition in the surrounding seawater.

C H A P T E R

I I

METHODS AND MATERIALS

2.1. STUDY SITE

Pilot experiments 1 and 2, and experiment 3 (Table 1) were carried out at Oudekraal on the west coast of the Cape Peninsula (Lat. 33°58'S ; Long. 18°21'E), the site of numerous studies on the kelp beds of the south western coast of South Africa (eg. Velimirov, et al, 1977 ; Newell, et al, 1980 ; Newell & Lucas, 1981 ; Linley & Newell, 1981 ; Dieckmann, 1980).
Experiments 4-8 (Table 1) were carried out at the 'Sea Fisheries Research Institute' (SFRI) in Sea Point/Cape Town, utilizing the filtered sea water available there (212 µm mesh size).

2.2. PLANT MATERIAL AND EXPERIMENTAL CONTAINERS

Ecklonia maxima plants were collected from just below low tide level of spring tides in both localities, cleaned of excessive epiphytes and kept in seawater containers for a period not exceeding 30 mins. between harvesting and the commencement of the experiment to prevent thalli from drying. The kelps used in the experiments were juvenile specimens selected to weigh between 0,1 and 0,25 kg (dry weight), corresponding to an approximate height of 1,5-2,5 m from the tip of the blades to the holdfast.

Containers used for storage and experimentation were commercially available 25 l plastic buckets that had been thoroughly cleaned with Contran and rinsed. In pilot study 1, a 100 l plastic container was also used. The buckets were filled with seawater (unfiltered or filtered) immediately prior to the start of the experiment. Depending on the size of the kelp assessed visually, 10, 12 or 15 dm^3 of seawater were used.

No.	Date	Type	Analyses	Venue
1	8-5-85	continuous	DON, polyphenols	Oudekraal, Cape
2	7-8-85	continuous	DON, DIN, DFAA	Oudekraal, Cape
3	28-10-85	continuous triplicate, A,B,C	DON, DIN, Bact. Activ. & Biomass	Oudekraal, Cape
4	21-3-86	4a discrete 4b cont. uptake	DON, DIN, UV, polyphenols DIN (enriched), FAA, DFAA	SFRI, Sea Point
5	20-5-86	discrete (dupl.)	DON, DIN, UV, polyphenols, Si, P	SFRI, Sea Point
6	6-6-86	continuous	DON, DIN, DOC, polyphenols	SFRI, Sea Point
7	8-8-86	cont. uptake	DIN (enriched), UV, polyphenols, P	SFRI, Sea Point
8	20-8-86	continuous	DON, DIN, DOC, polyphenols, P	SFRI, Sea Point

TABLE 1 : Summary of experimental dates and analyses conducted.

2.3. EXPERIMENTAL PROCEDURES

2.3.1. Pilot Studies

The pilot studies at Oudekraal (1 & 2 - Table 1) were carried out to establish the validity of the basic experimental design. The kelp was immersed upside down in a bucket of seawater for up to 120 minutes during which the water was sampled at 5, 10, 15, 30, 60, 90, and 120 minute intervals. Care was taken not to allow the cut surface at the holdfast to come into contact with the water to prevent wound exudates from entering the system. The kelp was continuously and gently agitated for the duration of the experiment. A sample taken just before the kelp was introduced into the system constituted the pre-exudation blank.

Another pilot study was set up with a piece of frond suspended over a beaker. The frond was rinsed, using artificial seawater (see Appendix 1b), at frequent intervals and the solution collected in the beaker. The exudate-containing solution was derivatized after 90 minutes and prepared for HPLC amino acid analysis (Section 2.7.5.1.). Quantification of DFAA on a unit mass basis of kelp was not carried out ; the experiment was designed to concentrate kelp-derived amino acids to characterize part of the DON liberated during exudation.

A pilot study designed to investigate UV-absorption of exudate is described in section 5.2.

2.3.2. Subsequent Experiments

The experiments that followed the pilot studies were of the same basic design but generally longer (up to 260 mins.). There were 2 sorts of experiments (Fig. 3) :

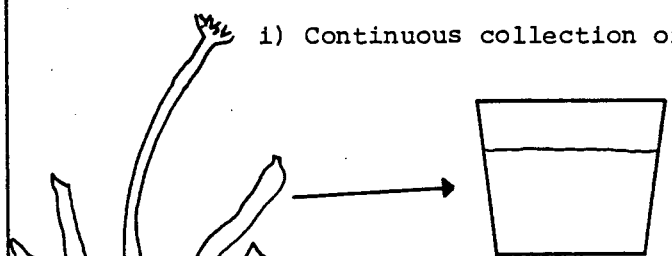
Continuous collection and discrete collection experiments. In

Fig. 3 : Graphic Representation of Experimental Design.

EXPERIMENTAL DESIGN

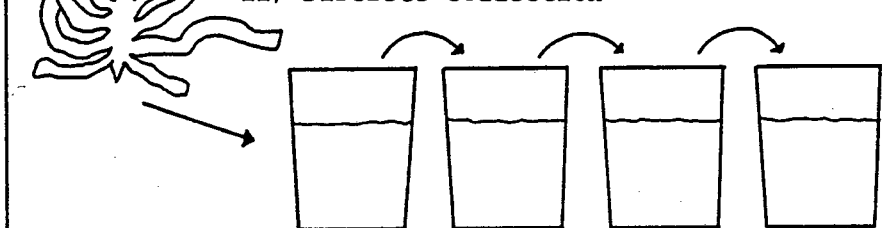
Experiments conducted either at Oudekraal (1985) using unfiltered seawater, or
at Sea Point (1986) using SFRI filtered seawater.

i) Continuous collection of exudate



Samples taken at o,5,1o,15,3o,6o,9o,12o
18o,(25o) min intervals

ii) Discrete collection



Samples taken at 1 : o,5,1o,15 ;
2 : o,5,1o,15 ; etc.

Samples filtered & frozen in liquid N_2

iii) All samples analysed for :

- NO_3^- and NH_4^+ using AutoAnalyzer (Mostert, 1983)
- Total N (DIN + DON) using an alkaline persulfate oxidation
- Polyphenols using modified Folin-Ciocalteu colourimetric test
- UV - absorbance on spectrophotometer

the continuous collection study, the seawater in the bucket was sampled for exudate or the disappearance of nutrient throughout the length of the experiment. The discrete collection mode involved the transfer of kelp to a different bucket every 15 minutes, with samples taken at regular 5 minute intervals. This method, which attempts to simulate a flow-through system, is a modification of that described by Carlson & Carlson (1984).

In the experiment of the 28-10-1985 (3) DON release was measured in 3 experimental as well as 3 'antibiotic' sets (section 3.2.3.) A control (C) was included to monitor DON levels without kelp. Containers were designated as A1, A2 and A3 (experimental) and B1, B2, B3 (antibiotic). A tritiated thymidine uptake study was included for A1 and C and bacterial counting for all sets (see section 2.8.).

The basic experimental design and subsequent analyses were modified in later experiments depending on data obtained in earlier experiments. A summary of experimental dates and analyses is given in Table 1.

2.4. NITROGEN AND PHOSPHORUS UPTAKE EXPERIMENTS

Uptake of N and P was studied as a function of the disappearance of the nutrient from the seawater. Phosphate uptake was studied under ambient PO_3 conditions only, while the uptake of ammonium-N and nitrate-N was monitored at both enriched conditions and ambient concentrations (Table 1).

Ammonium and nitrate in the form of NH_4NO_3 was added to the bucket in experiment 4b (21-3-86), while in experiment 7 (8-8-86) ammonium and nitrate were added separately to 2 experimental buckets in the form of ammonium chloride and sodium nitrate. The initial concentrations of N were much higher in exp. 4b than in

exp. 7 (approximately 100 and 30 µmol $N.dm^{-3}$, respectively). The enrichment experiments were of the continuous collection type, whereas uptake at ambient levels was studied by either continuous or discrete collection mode. An air-line was inserted during all the experiments for mixing. This experimental design (the depletion-of-nutrient in seawater) has been referred to as the "perturbation method" (Harrison & Druehl, 1981).

2.5. SAMPLING

Sampling was carried out using a 60 ml plastic syringe (Millipore), the volume distributed evenly into 3 plastic scintillation vials (Beckman or Packard type - cleaned and rinsed in a 5% HCl solution) using a disposable stick-on type Millipore 0,45 µm filter. Samples were immediately placed and stored in a liquid nitrogen kryo-container to halt any microbial degradation processes. Before processing, samples were allowed to defrost by placing them into a 0^{o} C coldroom overnight.

2.6. ANALYSIS OF INORGANIC NUTRIENTS IN SEAWATER

The chemical analyses for nitrogen, phosphorus and silicon were carried out on a Model II AutoAnalyzer at the laboratories of the SFRI in Green Point/Cape Town. Flow rates were those set by Mostert (1983) for this equipment.

2.6.1. Nitrate Analysis

Nitrate analysis of seawater was conducted using the principle of Copper-Cadmium (CuCd) catalysed reduction of nitrate to nitrite which produces a colour reaction with sulphanilamide and N-(1-naphtyl)-ethylenediamine dihydrochloride (EDDH-Cl). This method

is based on that described by Strickland & Parsons (1972) and adapted for automated equipment by Mostert (1983).

2.6.2. Ammonium Analysis

Ammonium / ammonia in seawater was analysed according to a modification of the phenol-hypochloride method (Solorzano, 1969 ; Strickland & Parsons, 1972), where the sample is treated in an alkaline citrate medium with NaOHCl and phenol in the presence of Na-nitroprusside. The blue indophenol formed with ammonia is measured in the AutoAnalyzer flow cell. Instead of using NaOHCl, sodium dichlorocyanurate acts as chlorine donor in this method (Mostert, 1983).

2.6.3. Reactive Phosphorus Analysis

Both inorganic orthophosphate and soluble organic phosphate (DOP) were measured by the phosphomolybdate method, where a blue complex of phosphate and molybdate tetrahydrate is formed in an acidic ascorbic acid medium (Mostert, 1983).

2.6.4. Silicon / Silicate Analysis

Silicate-silicon determination in seawater is based on that described by Mostert (1983). A blue heteropoly acid is formed as a result of the reduction of silicomolybdic acid.

2.7. ANALYSIS OF DISSOLVED ORGANIC MATTER IN SEAWATER

2.7.1. Determination of Total Organically Bound Nitrogen (DIN+DON)

The classic Kjeldahl digestion for the determination of total N in biological materials is less useful for seawater analysis, since the acid used in the digestion contains comparatively high amounts of nitrogen itself. This often results in high reagent blanks (Strickland & Parsons, 1972).

The method used in this study was an adaptation of that published by Nydahl (1978). In principle, oxidation of organic compounds is carried out by an alkaline potassium peroxodisulphate digestion. Nitrogenous groups are split from their carbon skeletons and a subsequent nitrate analysis results in a reading of total N content (Mostert, 1983). Rather than using either a tris buffer or hydrochloric acid to eliminate the magnesium hydroxide precipitate that is formed upon addition of the alkaline digestion medium, it was found that low speed centrifugation was a preferable method (see Appendix 1a).

[DON] was calculated as [total N] - [nitrate] - [ammonium].

2.7.2. Determination of Dissolved Organic Carbon (DOC)

Most methods for the determination of organic carbon in seawater involve either UV - absorption by organics or oxidation. Organic molecules are induced by acids or irradiation to break and oxidize to yield CO_2 which can then be measured (Wangersky & Zika, 1978).

The technique used in this work is based on the wet oxidation of DOC in a sulphuric acid medium through an excess of bichromate. Mercuric sulphate that is contained in the oxidant forms stable complexes with the chloride ions excluding them from causing

interferences with the method. The heat generated by the addition of H_2SO_4 (conc.) momentarily raises the temperature of the sample-oxidant mixture to boiling point facilitating the oxidation of organic matter. DOC is expressed as Chemical Oxygen Demand (COD) (Michel, 1972 ; Appendix 1c).

2.7.3. Analysis of Polyphenols in Seawater

A review of colourimetric techniques for the quantification of polyphenols in freshwaters has been published by Box (1983). The Folin-Ciocalteu method described was found to be unsuitable for marine samples. An adaptation of the Folin-Ciocalteu method was developed to prevent the precipitation of magnesium hydroxide in seawater (see Appendix 1b). Results were expressed as "phloroglucinol equivalents" (PGE) per unit volume (Carlson, 1982).

2.7.4. Ultraviolet-Spectrophotometric Analysis of Seawater

All UV-spectrophotometric measurements were carried out on a Pye Unicam 1800 Spectrophotometer. 40 mm quartz cuvettes were used and samples were read against distilled water blanks at 280 nm (Sieburth, 1969). A chart recorder was used for scans. Samples were read shortly after thawing to prevent oxidation and decolouring.

2.7.5. Amino Acid Analysis

2.7.5.1. Analysis of Seawater for Dissolved Free Amino Acids (DFAA)

The chromatographic separation of DFAA was carried out on a Beckman HPLC system, comprised of two Model 112 pumps, a 340 Organizer and a 421 Controller coupled to a Drew Scientific

Chromatography Interface linked to an Apple IIe terminal, for both gradient control and peak integration. Amino acids were detected in a Beckman 165 variable wavelength detector with a lamda setting of 256 nm. Separations proceeded in a 7 cm Altex Ultrasphere XL-ODS column with 3 um packing and a 25 cm Altex column with 5 µm packing.

The amino acid standards (Sigma) and samples were derivatized with 9-Fluorenylmethyl Chloroformate (FMOC-Cl) according to Einarsson, et al (1983). This type of precolumn derivatization is complete within minutes but resistant to bacterial degradation and the sample can be stored for months after derivatization (Garside, 1986).

2.7.5.2. Analysis of Kelp Tissue Material for Free Non-Protein Amino Acids (FAA)

After extracting the free unbound amino acids with ethanol (see Appendix 1d), amino acid analysis was carried out on a Beckman Model 120 C Amino Acid Analyser, using 0,2-0,5 ml of extract. Amino acids were separated on a 22 cm column of Beckman W2 spherical ion exchange resin using three lithium citrate buffers sequentially. The first buffer (0,02 M LiOH ; 0,15 M citrate) was adjusted to pH 2,83, the second (0,02 M LiOH ; 0,15 M citrate) to pH 3,7, and the third buffer (1 M LiOH ; 0,2 M citrate) to pH 3,75 (after Kedenburg, 1971). Once the amino acids were separated, postcolumn derivatization with ninhydrin followed, incubated at 100^{o}C and optical densities recorded on a Honeywell Electronik 16 logarithmic recorder and a Beckman 125 digital integrator. The pool sizes in µmol.cm^{-3} for each amino acid were calculated using the digital integrator readings corrected for by specific conversion constants for each amino acid, as determined from calibration runs.

2.8. MEASURING HETEROTROPHIC UTILIZATION OF KELP EXUDATE

Since exudation products often represent readily available organic carbon and nitrogen sources, rapid utilization of these substances by micro-organisms is often evident (eg., Brylinsky, 1977 ; Stuart, et al, 1981). A bacterial counting procedure coupled with a radioactive activity measurement technique was employed to determine heterotrophic utilization in the system.

2.8.1. Acridine Orange Direct Counts of Marine Bacteria (AODC)

Bacteria can be made visible using UV-fluorescence microscopy by staining with a fluorescent dye which is added to the water sample. The following method (AODC) is the most widely used method for assessing numbers of heterotrophs in aquatic and marine ecosystems (Linley, et al, 1981).

10 cm^3 seawater samples were collected in scintillation vials and fixed with 0,1 cm^3 of 25% glutaraldehyde. The samples were stained with Acridine Orange (Merck) prior to filtering onto Nucleopore polycarbonate filters (25 mm diameter, 0,2 um pore size) which had been stained by soaking in an Irgalan Black-acetic acid solution for 10 minutes to reduce background-fluorescence (Hobbie, et al, 1977).

Filtration was carried out on Millipore pads (AP 1004751) with a few drops of Photoflo to act as a surfactant. After filtering at low suction levels (<0,8 atm), the filter was transferred to a glass-slide and placed on a drop of low viscosity immersion oil. A Zeiss microscope fitted with an epifluorescence condensor, HBO 50W mercury burner, 455-500 band-pass exciter filter, 510 beam splitter and LP 520 barrier filter was used to view the bacteria at 1000 x magnification (Linley, et al, 1981).

2.8.2. Tritiated Thymidine Activity Study

Thymidine is precursory to bacterial reproduction and is actively taken up prior to replication. The uptake of radioactively labelled thymidine can therefore give an indication of the bacterial reproductive activity of the sample, which is a function of the availability of a suitable substrate (Fuhrman & Azam, 1982 ; Rivkin, 1986).

Water samples were taken from an experimental and a control exudation container, and tritiated thymidine (New England Nuclear) added. At certain intervals (see section 3.2.2) the incubation procedure was terminated by removing an aliquot from the incubation vial and subsequent addition of 0,1 cm^3 25% glutaraldehyde. All vials and tubes were stored on solid CO_2 until further processing (Fuhrman & Azam, 1982).

Later, each sample was filtered through 25 mm, 0,2 um Millipore GS filters. This was done in batches using a Millipore multiple filter holder connected to a vacuum pump. Together with 10 cm^3 of Packard 'Filtercount' scintillation cocktail, the filter was placed into a plastic scintillation vial (Beckman or Packard type). After the appropriate ^3H quench curve had been established, counting took place on a 460C Packard Liquid Scintillation Counter. Mathematical treatment and conversion of the results are given in section 3.2.

C H A P T E R

I I I

EXUDATION OF NITROGENOUS SUBSTANCES BY *Ecklonia maxima* AND THEIR HETEROTROPHIC UTILIZATION

3.1. INTRODUCTION

3.1.1. DON Release by Macroalgae

Most research on exudation has concentrated on DOC rather than on DON, mainly because DOC represents the bulk of substances liberated by algae and phytoplankton during exudation. However, most DON in seawater is still chemically uncharacterised and little is known about its biochemical properties (McCarthy & Carpenter, 1984).

The amount of DON is usually much larger than that of inorganic N (Jackson & Williams, 1985). The concentrations of DON range from 5 up to more than 30 umol $N.dm^{-3}$. Of this, only a small part constitutes the dissolved free amino acid (DFAA) pool ranging from 0,025 to 1,4 $umol.dm^{-3}$, with urea always being higher - up to 5 to 10 times in most cases (McCarthy, 1980). The finding that most of the DOM in seawater derives from the degradation and exudation phenomena of micro - as well as macroalgae (Mann, 1982), also applies to DON (section 1.2.). Because most of the work on exudation and extracellular production has focussed on DOC or total DOM, few estimates of DON release by algae have been put forward. A brief summary of selected papers dealing with macroalgal DOM exudation is presented in Table 1A and discussed.

Sieburth (1969) measured the production of DOM in the laboratory using Laminaria and Ascophyllum sp. Utilizing a quasi flow-through system (in order to curtail epiphytic utilization of the DOM) he arrived 40% of the total photosynthetic production being released into the surrounding water. This finding was surprising considering the apparent lack of an ecological advantage in a seaweed liberating up to 40% of its production.

Sieburth (1969) measured a variety of DOM classes such as nitrogenous, carbohydrate and polyphenol. An average 17,5 to 25% of the total DOM released consisted of nitrogenous matter, this having been measured by ninhydrin colourimetric tests. These high release figures were reflected in the findings of two Russian workers, Khailov & Burlakova (1969), who reported that a DOM equivalent of 39 % of gross photosynthetic production was released by a variety of brown algae (the 39% figure represents a pooled estimate). The UV-absorbance technique was used exclusively, subsequently found to be unreliable in converting UV-absorbance values to DOM concentrations (refer to section 5.1.). No discrimination was made between different classes of DOM.

Exudation can also be assessed quantitatively by deducting losses of photosynthates from annual carbon budgets. Of the net carbon assimilated throughout the year by Laminaria longicruris, 35% remained unaccounted for and was presumed to have been lost as DOM (Hatcher, et al, 1977). As was pointed out by Mann (1982), the figure of 35% does not distinguish between genuine exudation of healthy thalli and a loss of metabolites and mucilage that is produced in response to decomposition at the tips of the blades. Hatcher et al (1977) also commented on seasonal variation in DOM release. No mention was made of DON release since the study did not involve nitrogen budgets. Using a macroalgal C:N ratio of between 14 and 19 (Neill, 1976) it could be speculated that 1.5 to 3 % of the total annual production of Laminaria longicruris is released as DON. Johnston, et al (1977) calculated an exudation figure of 16% DOM, ~~also~~ for Laminaria ~~longicruris~~ Saccharina. This discrepancy between the two figures could well be due to seasonality. Additionally, exudation was measured directly using a variety of

TABLE 1 A

Table showing estimates for extracellular release in marine macroalgae

AUTHOR	YEAR	MACROALGA USED	MEASUREMENTS CONDUCTED	% of total PP released
Sieburth	1969	Laminaria sp Ascophyllum sp.	carbohydrates polyphenols	4o
Khailov & Brlakova	1969	Variety of browns	UV-absorbance	39
Hatcher, et al	1977	Laminaria sp.	carbon budget	35
Brylinsky	1977	Variety of browns	$^{14}CO_2$	1-3
Johnston, et al	1977	Laminaria	DOC techniques	16
Frankboner & de Burgh	1977	Macrocystis sp.	$^{14}CO_2$	o.oo2
Carlsson & Carlsson	1984	Ascophyllum, Fucus	DOC ; UV- absorb. polyphenols	2-5

DOC techniques rather than calculating back from a C-budget balance sheet as Hatcher, et al, had done. Newell & Field (1983) estimated energy flow through South African kelp-beds from C:N ratios.

Extracellular release has been estimated using radioactive tracer techniques. Working on Macrocystis integrifolia Bory, Fankboner & de Burgh (1977) came to the conclusion that DOM production did not amount to more than 0,002 % of the total carbon fixed and that the high release values obtained by earlier workers were the result of either experimental artifacts or physiological stress. If their estimates are correct, DOM - and DON - release during exudation is minimal. On the other hand, Brylinsky (1977) arrived at a DOM release figure of 1 - 3 % using the same technique but working on different seaweeds. Recently, Carlson & Carlson (1984) determined exudation rates by measuring DOC and polyphenol content in the surrounding seawater. Two seaweeds, Ascophyllum nodosum (L.) Le Jol. and Fucus sp., were used in continuous and discrete collection studies. Average values for exudation were calculated to constitute 2 - 5 % of daily production by the algae. DON release was not measured.

It can be seen that DON measurements have been largely neglected, which is surprising considering the relative ease with which total nitrogen (DON plus DIN) can now be measured. Although DOC of macroalgal origin is much more prominent in exudates than is the DON, this source represents an important input of nitrogen into the ecosystem and can be utilized by heterotrophs and producers alike (McCarthy, 1980).

3.1.2. Dissolved Free Amino Acids (DFAA) as Part of the DON Pool

It has been estimated that the replacement time of DOM in the oceans can be up to thousands of years in some cases (Williams, et al, 1976). However, turnover rates of DFAA are much more rapid. This is mainly due to the selective removal process of organic substances in the oceans (Ogura, 1975). Amino acids can be derived from a variety of sources. They are formed as the product of leaching and exudation of primary producers or generated as a result of heterotrophic activity. Amino acids in the marine environment are immediately subject to consumption by heterotrophic bacteria (Amano, et al, 1982). In extremely nitrogen depleted waters amino acids are also taken up by phytoplankton (Lee & Bada, 1977).

Amino acid composition and their respective concentrations can be of value for mainly two reasons : Firstly, bacterial preference for individual amino acids can be determined (Dawson & Gocke, 1978). Secondly, by studying the profiles of amino acids in seawater it should be possible to detect trends linking the original source of the amino acids to those detectable in the water (Dawson & Gocke, 1978). For example, the amino acid alanine is prominent in tissue extracts of kelp and is often found to be present at higher levels in coastal than in mid-oceanic waters (Lee & Bada, 1975). Clark, et al, (1972) identified the following amino acids in a Southern Californian kelp bed : Glycine and serine were most common with glutamine, asparagine and alanine being slightly less. The validity of these samples appears doubtful today in the light of the inaccurate techniques that were employed (Garrasi, et al, 1979). Wide fluctuations in the DFAA concentrations are

brought about by heterotrophic utilization, since amino acids serve not only as a carbon but also as a nitrogen source. Therefore, although the production of amino acids by primary producers and decomposers may be reasonably high, utilization and subsequent reabsorbance are accountable for a high flux and variability in detection.

3.1.3. Heterotrophic Utilization of Kelp Exudate

Bacteria and other marine heterotrophs are instrumental in regenerating and remineralizing inorganic nutrients (Newell & Field, 1983). Material present in the combined POM and DOM pools are subject to decomposition by heterotrophs and converted to either bacterial biomass or lost as heat from the system (Valiela, 1984). Depending on the type of compound, microbial attack can lead to rapid depletion or slower rates of degradation. Sugars and proteins are rapidly degraded, while other substances (cellulose, waxes, lignins and phenolics) are less easily degraded leading to a change in composition of the POM and DOM pools (Velimirov, et al, 1981).

Considering bacterial decomposition rates, Andrews & Williams (1971) found pronounced seasonal fluctuations in the oxidation of certain seawater soluble substrates in waters of the English Channel. Most markedly, this could be observed in the case of glucose. In winter, less than 1 % of the total available glucose (TAG) was utilized; this figure increasing to between 20 and 50% TAG utilization per day. The same trend was noted in the DFAA pool : values being low (usually around 1%) in winter, increasing to approximately 4 - 10 % of DFAA utilization per day in late summer. Ogura (1975) noticed decomposition of DOM to proceed in two ways : there was a rapid decrease in coastal seawater DOM during the first few days followed by a

much slower degradation process thereafter. Ultimately, 10-30% of phytoplankton derived DOC was measured to be taken up rapidly, primarily because of its lability. 20-30% of exudates of macroalgae were found to be assimilated by heterotrophic micro-organisms within only 2 hours (Brylinsky, 1977). In a study on South African kelp systems, Stuart, et al (1981) determined 29 % of the total DOC released from kelp, measured as mucilage, to be converted into bacterial biomass, while only 11 % of the particulates were effectively used by bacteria.

Assuming a total annual production of 14583 x 10^3 kg of mucilage being liberated by a 700 ha Cape Peninsula kelp bed, Lucas, et al (1981) estimated this amount of mucilage to be able to support approximately 3 x 10^5 kg bacteria and 3 x 10^4 kg of flagellates and ciliates. This would indicate a high efficiency rate of kelp material being converted into bacterial biomass. Indeed, it has been suggested that up to 30% of DOM is converted into bacterial biomass during the summer months and around 14 % in winter. Data extrapolated by Newell & Lucas (1981) indicate that the annual production of bacteria in the water column corresponds to approximately 11,5 $g.m^{-3}.yr^{-1}$ rising to 18,1 $g.m^{-3}.yr^{-1}$ during summer. Thus, energy flow through a kelp - dominated system is largely mediated by bacteria which provide the link between primary producers and consumers (Field, et al, 1980).

3.1.4. Aims

The aims of this section of the study were :

To quantify exudation of organically bound nitrogen in continuous and discrete collection mode and to characterize amino acids present in seawater as a result of exudation. A bacterial count

and activity study were also carried out to provide a measure of heterotrophic utilization during extracellular release.

3.2. MATERIALS AND METHODS

DON was determined as detailed in section 2.7.1 ; ammonium and nitrate analyses according to section 2.3. The analysis of DFAA in seawater was conducted as outlined in section 2.7.5.1. Measurement of heterotrophic utilization of kelp exudates was carried out using an Acridine Orange direct counting procedure (AODC) and bacterial activity was monitored by a tritiated (^3H) thymidine method (section 2.5.2.).

3.2.1. AODC Data Conversion

Bacterial density (cells.dm^{-3}) can be calculated from the following expression, regardless of different size classes (Hobbie, et al, 1977) :

$$N = \frac{S \times 10^6 \, n}{s \times V} \quad \text{cells.ml}^{-1}$$

where S = working surface of filter in mm^2;
s = surface area of field of observation in μm^2;
V = volume of sample filtered in ml;
n = average number of cells per field.

These data can be further manipulated to arrive at direct biomass results of bacteria from densities per unit volume. To determine biomass the different uptake potentials of the different bacteria must be considered. Large bacteria (rods) are considered to take up more metabolites than small ones (cocci). Each group's contribution to the total bacterial flora must be taken into consideration.

Biomass can be calculated from the following equation (Painting,

et al, 1985 :

$$B = N \times Sg \times Vc \times 10^{-7}$$

where B = Biomass in $pg.dm^{-3}$
N = Bacterial density (separate according to group) in cells per cubic mm
Sg = specific gravity of bacterium (=1,1)
Vc = cell volumes in cubic µm :

small cocci =	0,009
large cocci =	0,142
small rods =	0,198
large rods =	0,672

3.2.2. ^3H - Thymidine Incubation Conversion

The residual radioactivity in the water (ie. not taken up by bacteria) was measured on a scintillation counter (section 2.5.2.) and values are expressed as disintegrations per minute (DPM). The lower the DPM value, the more ^3HT has been taken up by bacteria and retained by the Nucleopore filter, ie. the higher is the bacterial activity. The data obtained per incubation at every sample time were plotted (versus time) and their slopes calculated using a linear regression formula. Values for blank incubations can be subtracted from cell counts but failing to do so will not affect the numerical value of the regression slope. The slope consisting of subsample determinations of the incubations becomes steeper as more radioactive substrate is assimilated. The steepness of any particular slope will thus indicate uptake velocities of thymidine and hence activity.

Data were manipulated further as follows :

a) Moles of thymidine incorporated per minute
$= DPM.min^{-1} \times (SA)^{-1} \times C$

where $DPM.min^{-1}$ = Slope from DPM versus time graph

SA = Specific activity of isotope ($4,9 \times 10^4$ $Ci.mol^{-1}$)

C = numbers of curies per DPM($4,5.10^{-3}$)

b) Moles incorporated per litre per day
= $mol.min^{-1}$ x (Volume filtered in dm^{-3})
x (minutes per day)

These results can be used to express bacterial activity when macroalgal exudates are present as substrate. The ^{3}HT incubation study was only carried out on one experimental bucket (A1) and the control for practical reasons.

3.2.3 Addition of Antibiotics to Prevent Heterotrophic Activity

1 g of each Chloramphenicol, Erythromycin and Tetracycline were dissolved in 100 cm^3 of a 20% ethanol solution. Per dm^3 of seawater 7,5 cm^3 of stock solution was added to prevent heterotrophic activity (Stuart, 1982).

3.3. RESULTS

3.3.1. Liberation of Dissolved Organic Nitrogen

3.3.1.1. Pilot Study Results

Pilot study 1 (8-5-85) :

The amount of total N (DON+DIN) was seen to approximate to 2 mg $N.dm^{-3}$ with no significant accumulation of kelp-derived organics (Table 2). More total N was present in the larger bucket (80 dm^3) at the start and end of the experiment.

Pilot study 2 (7-8-85) :

From an initial total N value of 10-12 µmol N.dm^{-3} (= 5,25 µmol N.dm^{-3} DON) there was a decrease to total N values to around 6-7 (= 1-2 µmol N.dm^{-3} DON), followed by a peak at 30 mins (Table 2). After 60 mins. there was an accumulation of total N and DON (value at 120 mins.: 16,97 and 11,34 µmol N.dm^{-3}, respectively).

Table 2 : Total N values for pilot study (8-5-85) in mg N.dm^{-3}

t(mins)	[N]-small	[N]-large bucket
0	1,620	1,961
5	1,960	1,876
10	1,870	2,630
15	2,210	1,870
30	1,960	1,710
60	1,620	2,040
90	1,660	2,040

Total N data for pilot study (7-8-85) in µmol N.dm^{-3}

t(mins)	[Ammonium]	[Nitrate]	[Total N]	[DON]
0	2,12	4,58	11,95	5,25
5	1,65	3,41	10,3	5,24
10	4,52	4,06	6,32	-
15	2,68	2,46	6,4	1,26
30	2,60	4,43	15,76	8,73
60	1,76	3,54	9,94	4,64
90	2,18	4,28	11,16	4,70
100	1,65	3,78	12,61	7,17
120	2,05	3,58	16,97	11,34
B	1.6	0.19	2,47	0,68

3.3.1.2. Continuous Collection Results

Data are presented as Total N in Fig. 4. for the experimental sets (Data in Appendix· 2). There was little change in the concentration in the 5 min. sample from that observed initially (10-15 µmol $N.dm^{-3}$. During 5-10 min. interval, values in all 3 sets increased sharply to between 48 and 54 µmol $N.dm^{-3}$ in A1 and A2 and to approximately 30 µmol $N.dm^{-3}$ in A3. After 15 min. all three sets had again decreased in concentration to almost initial levels. Between 30 and 60 mins, A1 and A3 increased in total N concentration to 64 and 51 µmol $N.dm^{-3}$, respectively, while A2 did not change significantly between 30 and 90 mins. After 90 mins., at which all 3 were similarly low at 11 µmol $N.dm^{-3}$, a further increase took place in all A to between approximately 40 and 50 µmol $N.dm^{-3}$ at 120 mins., at which point in time the kelp was taken out but the system monitored further for another 2 hours. As a result of the removal of the kelp, the trends of total N had become successively more and more out of phase. A1 decreased slowly to the background level until the termination of the experiment. Contrary to A1, both A2 and A3 showed upward fluctuations ; however, they were not synchronised.

This trend was reflected by the standard errors that were calculated. After 120 min (SE = 2,90), the values for standard errors increased to 12,5 at 150 mins. and then remained constant at around SE = 13-14 thereafter in A. They were uniformly low in the B set (See Fig. 6. in Appendix 3).

In the control, concentration values were approximately within the range of 10 µmol $N.dm^{-3}$.

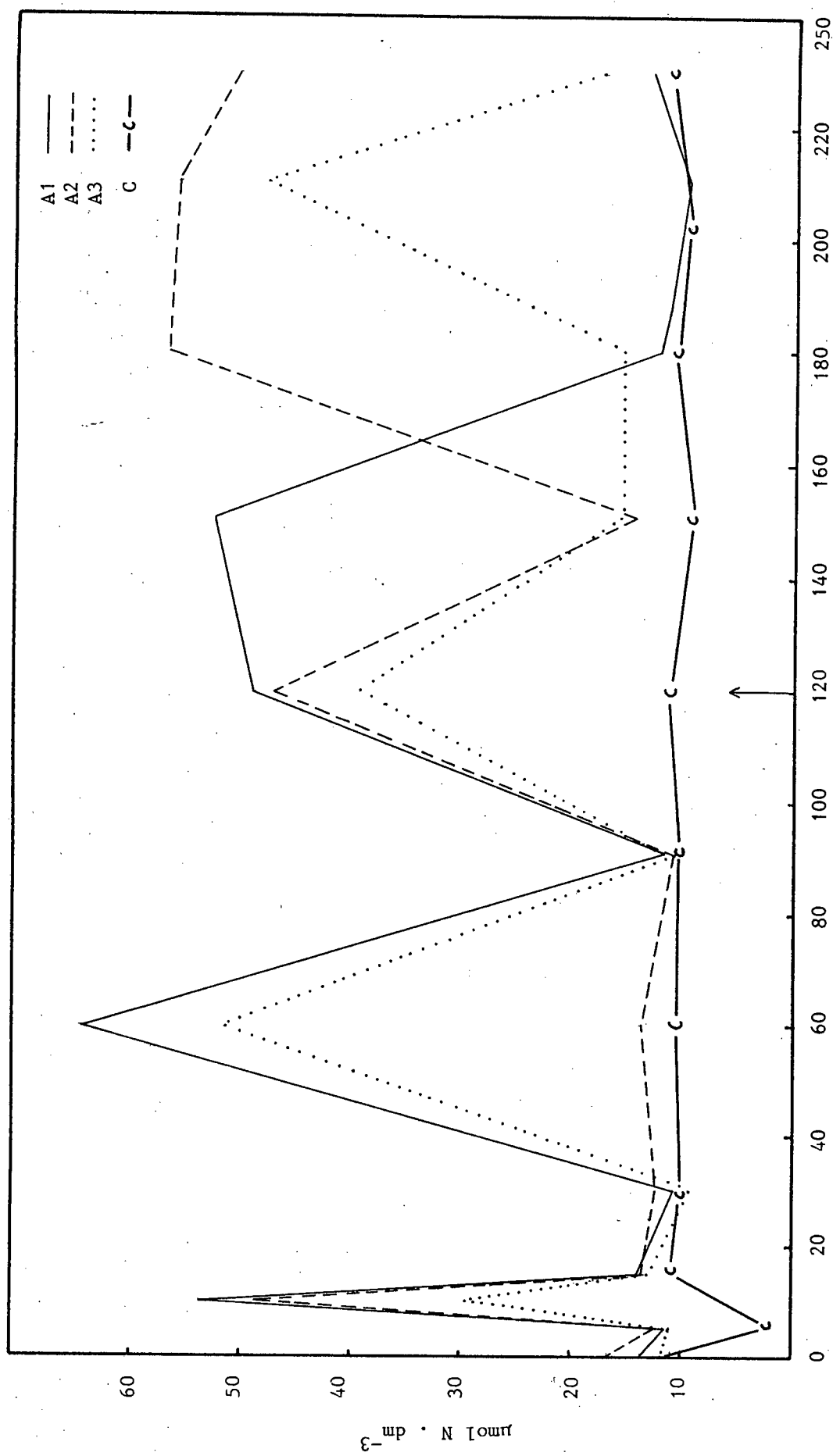


Fig. 4 : Exp. 3 ; DON measurements of experiment and control (arrow = kelp taken out)

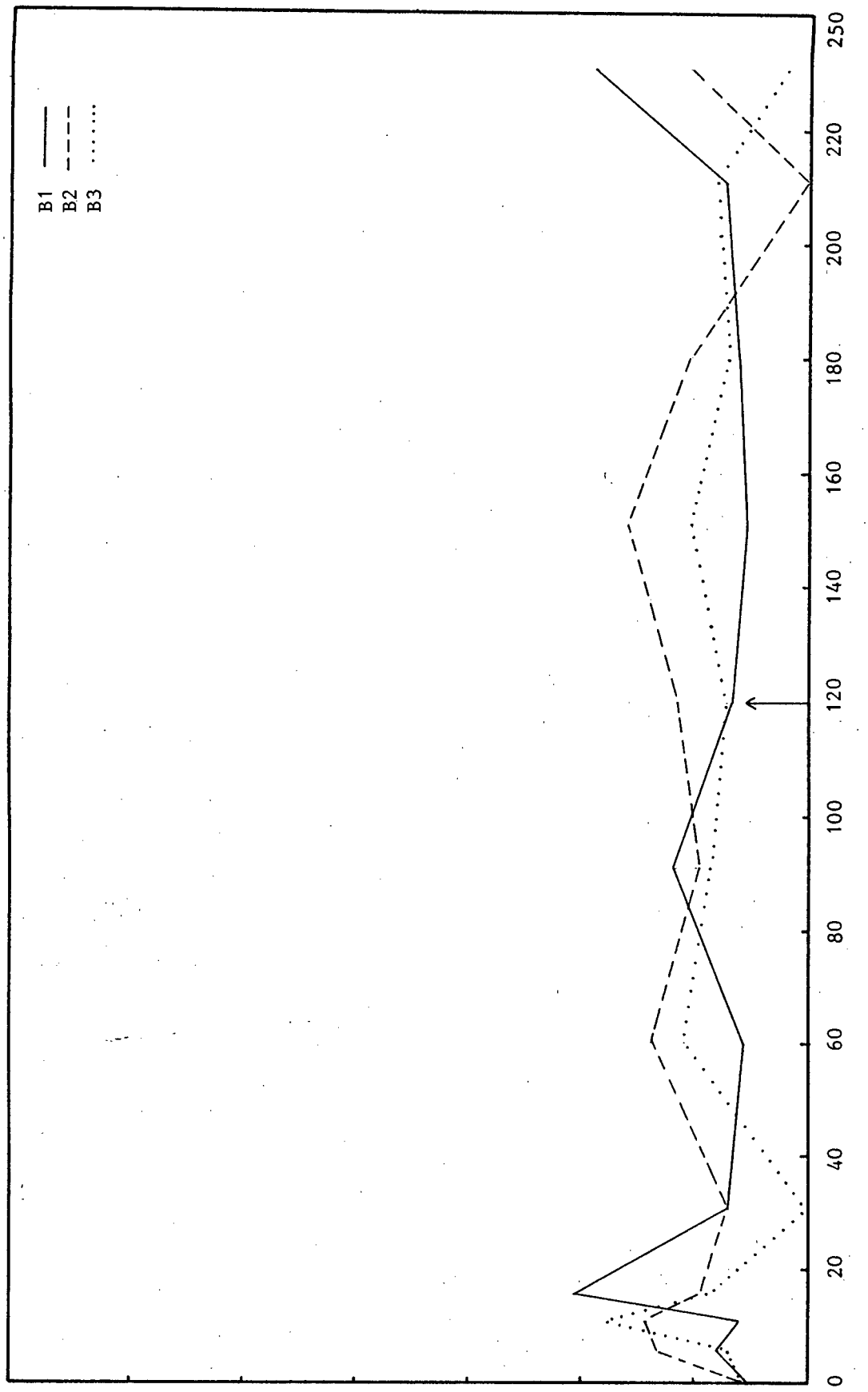


Fig. 5 : Exp. 3 ; DON measurements of antibiotic containers

All concentration values for B1, B2 and B3 were lower than those for A1, A2 and A3 at all times (Fig. 5, Appendix 2). There was increase from a lower blank (+- 3 µmol $N.dm^{-3}$) to approximately 6-7 umol $N.dm^{-3}$ within 10-15 mins. Between 15 and 30 mins. there was a decrease in total N in all sets, comparable to that observed in A. No change of DON took place after the kelp was removed at 120 mins.

The N-enrichment experiment 4b was analysed for total N as well as nitrate and ammonium (section 2.6.). It was found that higher levels of DIN interfered with the digestion procedure, possibly because of thelimited oxidation potential of the peroxo - solution.

In experiment 6 the following pattern of DON was observed (Fig. 7 and Appendix 2). From an initial level of 31 µmol $N.dm^{-3}$, the concentration peaked at 40 µmol $N.dm^{-3}$ within 10 mins. After 120 mins. DON levels had decreased further to 21,5 µmol $N.dm^{-3}$ and rose again to 26 µmol $N.dm^{-3}$ after 250 mins. Accumulation of organically bound N occurred between 120 and 250 mins. corresponding to a net increase of 2,27 µmol $N.dm^{-3}.hr^{-1}$.

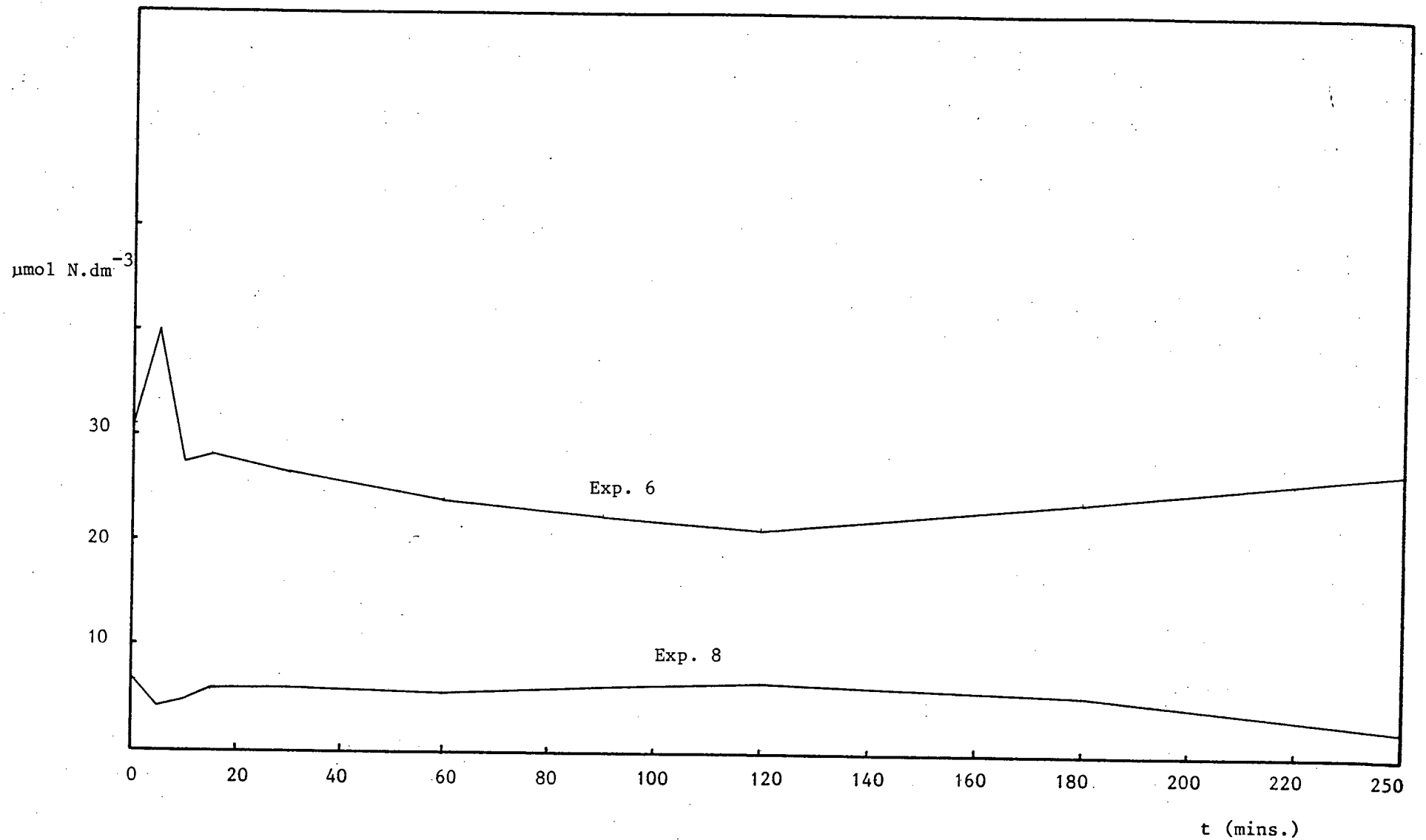


Fig. 7 : Exp. 6 & 8 ; DON measurements

A similarly low value of net DON output was observed in experiment 8. Apart from an initial disturbance in the level of total N, the concentration in the system remained constant and eventually decreased to a low level of approximately 11 - from an initial 17,1 µmol $N.dm^{-3}$ (Fig. 7. and Appendix 2). Because of the concomitant uptake of nitrate in the system (see section 4.3.) there was a slight increase in the DON content not visible from the total N trends. Between 5 and 120 mins., DON rose from 4,07 to 6,88 µmol $N.dm^{-3}$ which corresponded to a net accumulation of 1,47 µmol $N.dm^{-3}.hr^{-1}$ (tentatively calculated).

3.3.1.3. Discrete Collection Results

Fig. 8 shows the exudation pattern of DON obtained by discrete collection in exp. 4a. In the first discrete sample set, the concentration of DON reached a maximum of 67 µmol $N.dm^{-3}$ after the first 10 mins.; this value was also the highest concentration recorded during the experiment. The peak in the second set appeared earlier - between 5 and 10 mins. and had a DON value of 66 µmol $N.dm^{-3}$. The individual release peaks recorded thereafter were successively lower : 54 and 39 µmol $N.dm^{-3}$ for set 3 and 4, respectively. No release of DON was observed in set 5. Set 6 was characterized by a sudden pulse of DON of magnitude 48,6 µmol $N.dm^{-3}$. Set 7 was similar to 5 by its apparent lack of release.

Experiment 5 was performed in duplicate (Fig. 9),including a determination of 3 blank digestions (using the filtered seawater at the SFI) resulted in a mean value of 7,15 µmol $N.dm^{-3}$ of DON as a background value. The first 2 sets showed a weak accumulation with DON concentration values lower than 15 µmol $N.dm^{-3}$. Strong pulses of DON occurred in set 3 and 4 where values of >100 µmol $N.dm^{-3}$ were reached. However, there was no

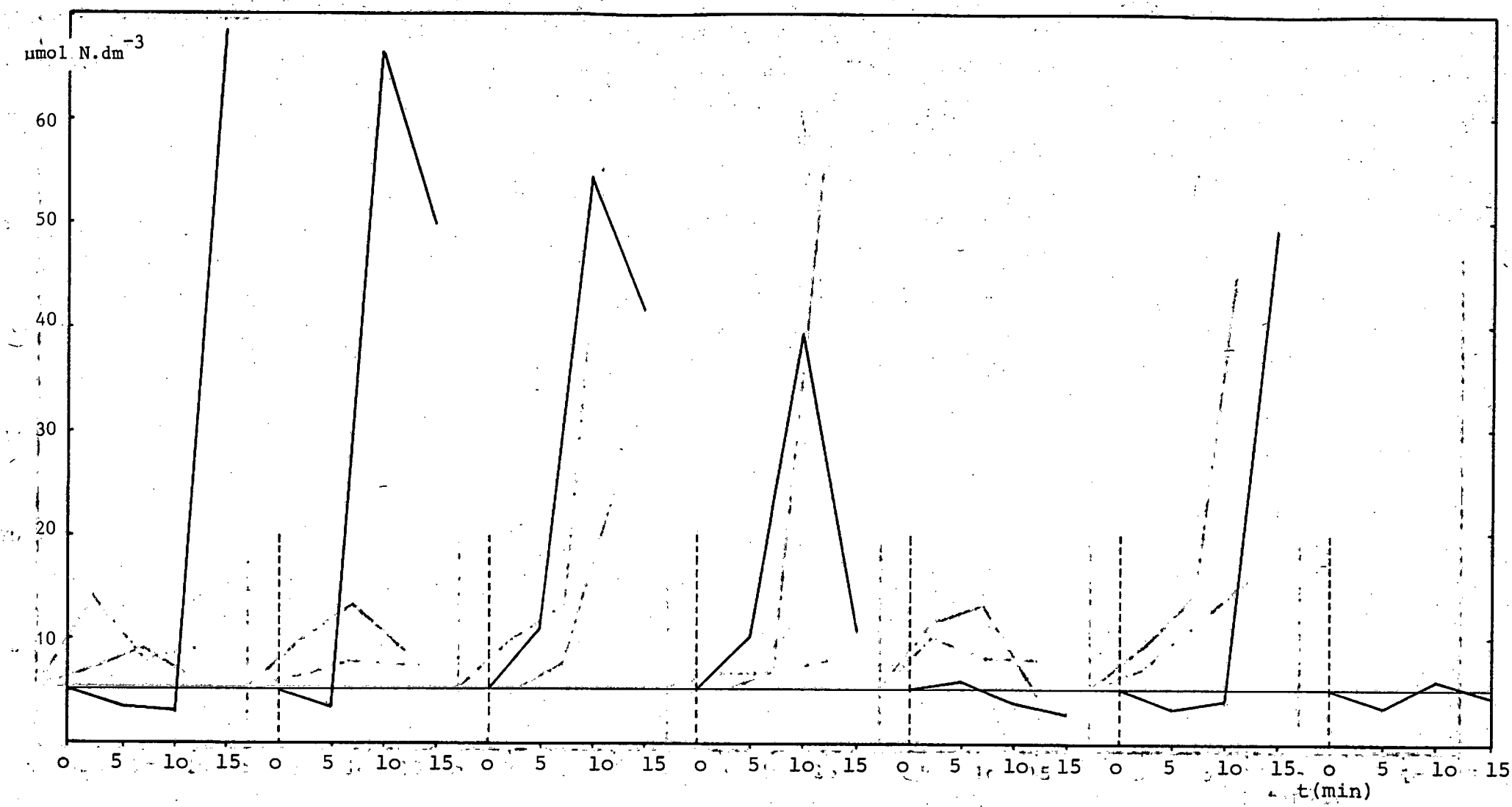


Fig. 8 : Exp. 4a ; DON measurements (discrete coll.). Baseline at 6 umol $N.dm^{-3}$

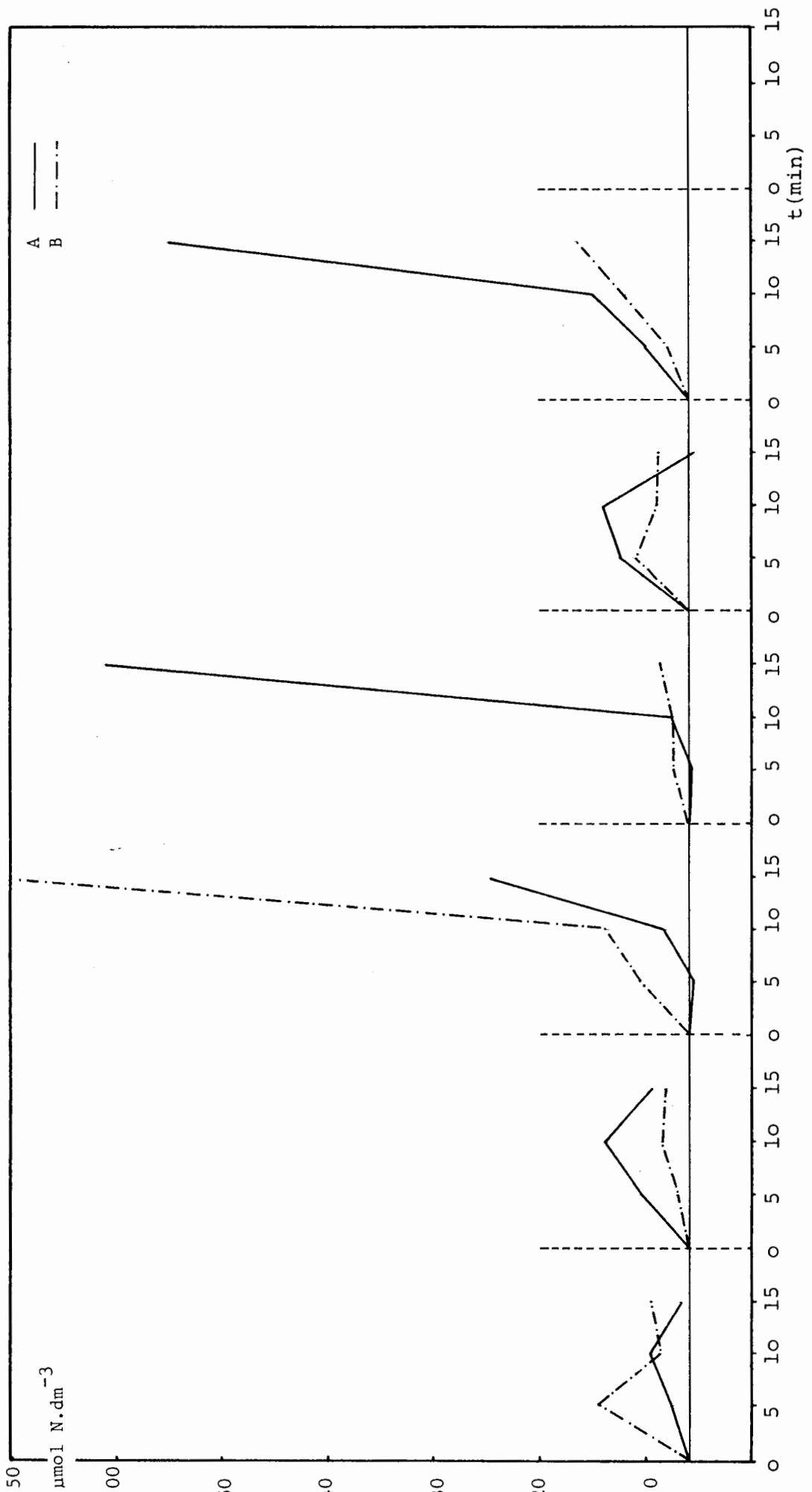


Fig. 9 : Exp. 5 ; DON measurements (discrete coll.). Baseline at 6 umol N.dm^{-3}

trend or replication noticeable. No release was observed in set 5 (representing time 60-75 mins. of experiment) in either kelp. A pulse of DON took place only in one replicate in set 6. Except for sets 1, 2 and 5, where little or no DON was released, duplication did not appear to indicate any reproducible trends. The rapid increases in DON exudation in sets 3, 4 and 6 represented release rates of 8,05; 3,57 and 4,325 mmol(kg dwt)$^{-1}$ within 5 minutes.

3.3.2. Flux of Dissolved Free Amino Acids (DFAA) during Exudation in Ambient Seawater

Pilot study :

The amino acid profile of the pilot study described in 2.3.1. is shown in Fig. 10. The following DFAA (in descending order of concentration) were detected : Alanine, arginine, glycine, glutamic acid, cysteine, aspartine and histidine. From a run of standards it could be determined that concentrations ranged from 12,6 µmol N.dm^{-3} (alanine) to 2,5-3 µmol N.dm^{-3} (aspartine), which was the detection limit in this case.

3.3.2.2. Detection of DFAA in Continuous Collection Experiments

During exudation study 2, water samples were analysed for amino acid profiles at 0, 10, 30, 60 and 120 minutes. (refer to Table 3). Profiles showed varying concentrations of the following unidentified amino compounds : A, B, C, and D. These peaks were unaccounted for when comparisons were made with the amino acid standard runs.

Table 3 presents the concentrations of the individual DFAA over time. Comparing the runs, a number of trends were visible :

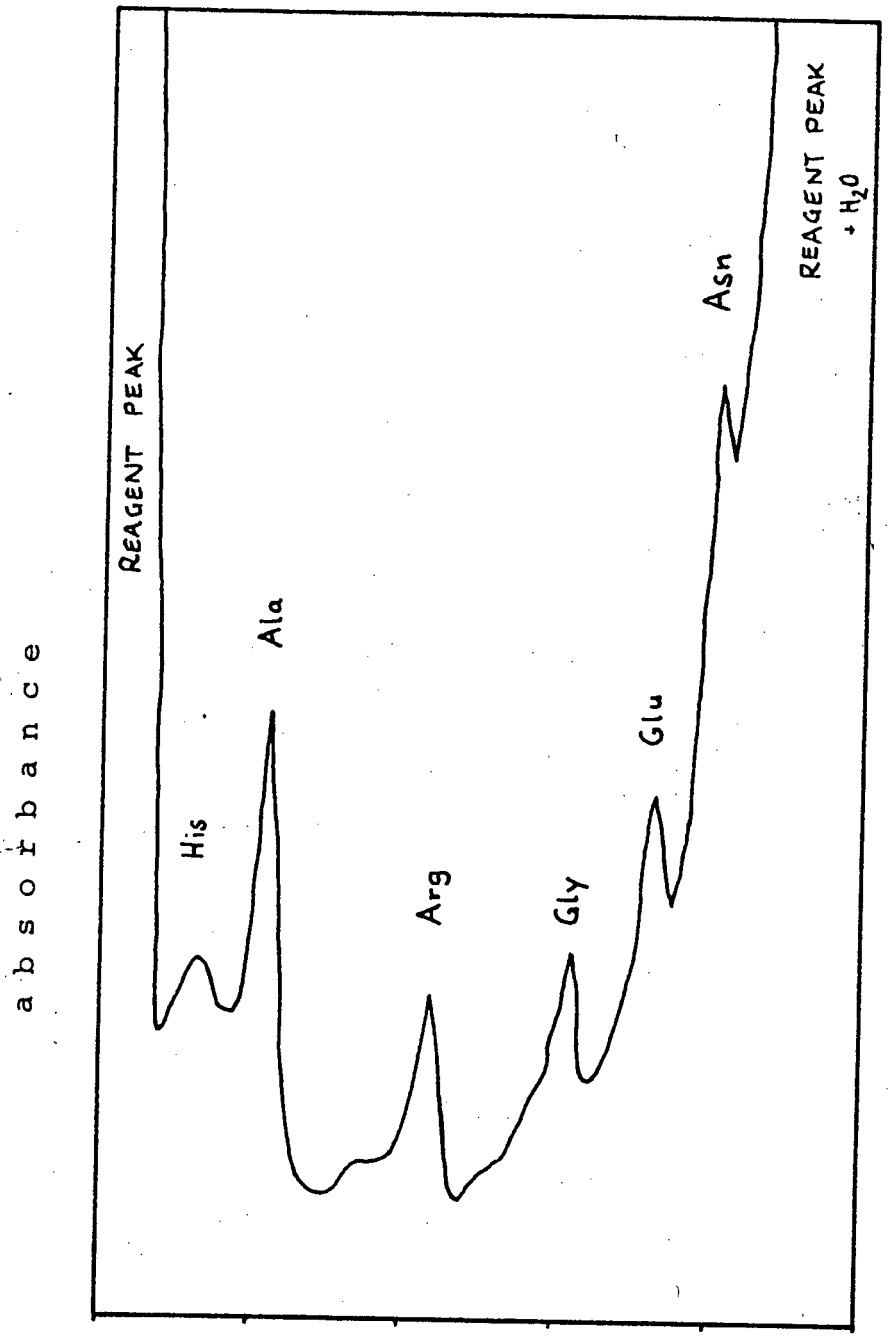


Fig. 10 : Qualitative HPLC run of DFAA pilot study : HPLC amino acid profile (axes arbitrary).

Amino acid	0	1o	3o	6o	12o mins.
alanine	1,48	1,98	1,26	1,36	1,75
aspartic acid	2,21	3,21	3,3o	3,47	3,21
glutamic acid	1,21	2,o9	1,43	1,48	1,88
glycine	1,o5	1,32	1,oo	1,o3	1,38
lysine	1,15	1,33	o,47	o,32	1,25
histidine	-	o,51	-	o,21	o,48
arginine	-	o,29	o,31	o,38	o,38
valine	-	-	o,16	-	o,26

TABLE 3 : HPLC analysis of DFAA in exudation study 2.
Data given in µmols . dm^{-3}

a) There were fewer DFAA within the detection range of the HPLC at the beginning than towards the end of the experiment.
b) Concentrations of DFAA were generally higher towards the end of the experiment.
c) All detected amino acids (excluding unidentified B, C, and D) were seen to peak at 10 mins (no quantitative determination possible due to lack of suitable standard).
d) Minor quantitities of other amino acids were detectable after 120 mins. when they had accumulated sufficiently. These included: leucine, isoleucine, phenylalanine, valine and methionine.

Analysis of seawater for DFAA was carried out for experiment 4b Table 4). The concentration values of the more important amino acids were generally lower than observed in the previous experiment. Other trends included :

a) Alanine decreased after an initial value of 0,7 $\mu mol.dm^{-3}$ and remained at approximately 0,6 $\mu mol.dm^{-3}$.
b) Glycine was detectable at 5 mins.; only traces of it were recorded at 0 and 10 mins. There was a return to 0,12 $\mu mol.dm^{-3}$ and at 180 mins had reached a peak value of 0,25 $\mu mol.dm^{-3}$. After 24 hours glycine was present at 0,45 $\mu mol.dm^{-3}$.
c) Cysteine and lysine peaked during the first 15 mins. of the experiment. Similarly, aspartic acid and arginine peaked during the first 10 mins. and returned to a level of 0,65 and 1,1 $\mu mol.dm^{-3}$, respectively. Aspartic acid and arginine were found to be the most common DFAA, followed by alanine.
d) After 24 hr, glutamic acid and serine, together with arginine and alanine were present in the exudate in high quantities (8,37; 7,6; 9,64 and 9,38 $\mu mol.dm^{-3}$, respectively). However, aspartic acid had disappeared from the profile.

Amino Acid	0	5	10	15	30	60	120	185	24h
arginine	0,89	1,20	0,89	1,28	1,23	1,32	1,33	1,27	1,93
alanine	0,72	0,51	0,58	0,61	0,59	0,58	0,55	0,55	1,88
cysteine	0,31	0,31	0,34	0,14	t	t	0,20	-	0,48
aspartic acid	-	0,73	0,08	0,55	0,59	0,75	0,50	0,79	0,04
aspartine	t	t	0,05	t	t	t	0,06	0,07	0,06
lysine	0,29	0,22	0,07	0,06	0,13	0,16	0,11	0,11	>5 ?
glycine	t	0,10	-	0,13	0,70	0,11	0,11	0,25	0,45
glutamic acid	t	-	0,07	0,04	0,09	0,06	0,07	0,12	1,67
serine	t	-	0,07	t	t	-	-	0,03	1,52
tyrosine	0,10	0,66	0,14	0,35	0,32	0,52	0,22	0,70	0,19

TABLE 4 : Exp. 4b. Concentrations of DFAA in N-enrichment experiment.
(conc. in umol.dm-3)

(t = trace amount)

3.3.3. Monitoring of Dissolved Organic Carbon (DOC) during Exudation.

DOC was measured on samples obtained in experiments 6 & 8. Fig. 11 displays the concentration of DOC as measured as chemical oxygen demand (COD) expressed in mg $O_2.dm^{-3}$. There were two initial pulses of DOC at 5 and 15 mins., after which COD decreased to almost baseline again (8 mg $O_2.dm^{-3}$). There was a strong increase in COD between 60 and 90 mins (9-34 mg $O_2.dm^{-3}$), followed by a removal of oxidizable substances (18 mg $O_2.dm^{-3}$) and a final equilibration at around 26 mg $O_2.dm^{-3}$, representing 3,25 times the COD that was measured in the pre-exudation blanks (0).

COD was lower in experiment 8. Background concentration of oxidizable substances was reduced from 5,5 to 1 mg $O_2.dm^{-3}$ COD, and increased slowly to a maximum value of 11 mg $O_2.dm^{-3}$. From there on COD was declining and reached a mean value of 8,5 mg $O_2.dm^{-3}$ - 1,54 times the original ambient level.

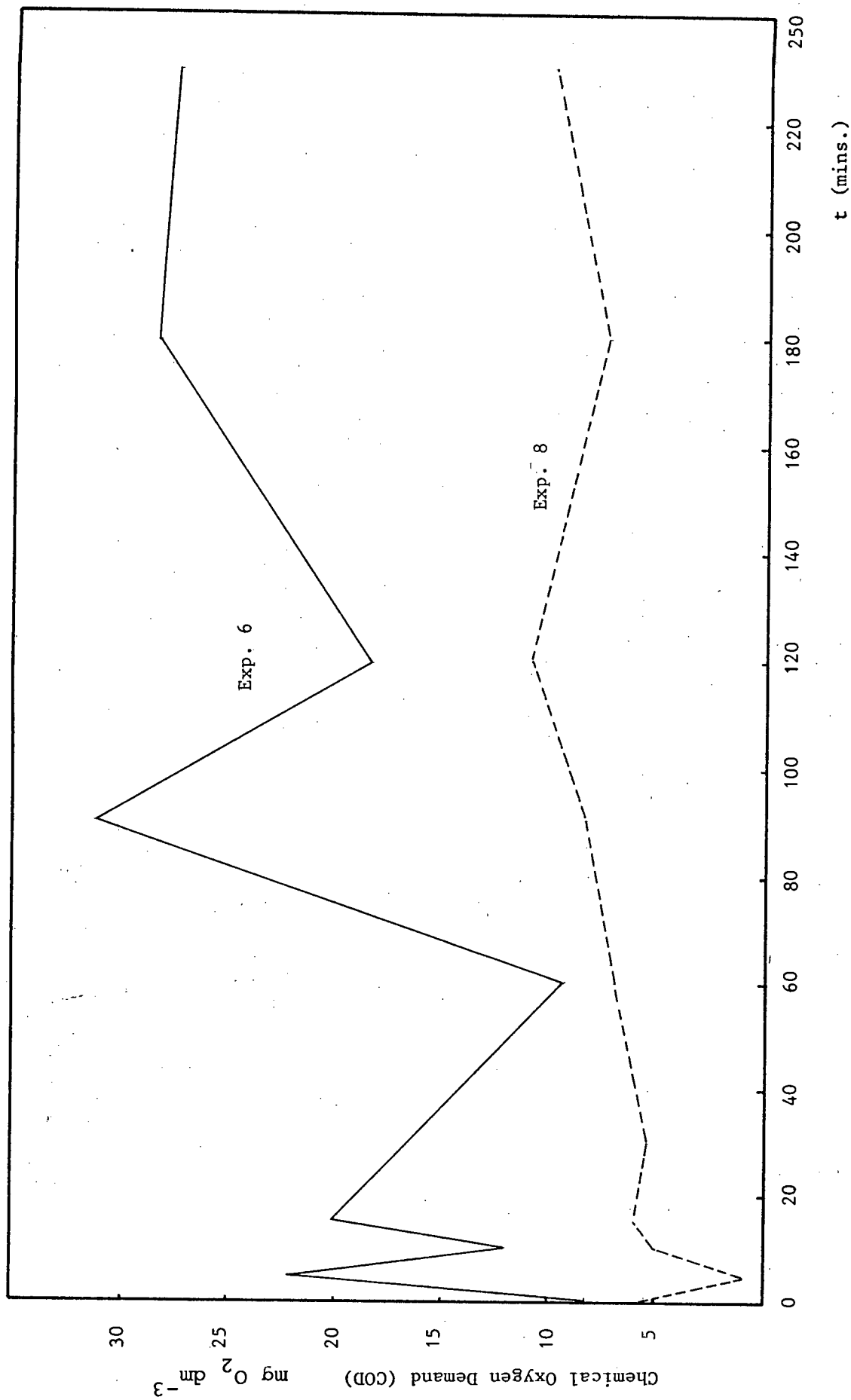


Fig. 11 : Chemical Oxygen Demand (COD) measurements in exp. 6 and 8 indicating DOC quantities.

3.3.4. Heterotrophic Utilization of Exudation Derived DOM.

3.3.4.1. Acridine Orange Direct Counts (AODC)

Bacterial biomass values in picogram per cubic decimeter of seawater are shown in Table 5. Pre-exudation bacterial biomass values ranged from approximately 7 to 20 $pg.dm^{-3}$. With the exception of A1, biomass values peaked at 5 mins and decreased subsequently. A3 showed a large bacterial population in the experiment at 15 mins. Biomass was determined as 136 $pg.dm^{-3}$. The decrease in bacterial biomass and numbers was common to all sets between 30 and 60 minutes. Bacterial biomasses appeared to remain constant or decrease after the kelps had been taken out. Values for A1, A2 and A3 at the termination of the experiment were 19; 6,3 and 36,5 $pg.dm^{-3}$,respectively.

Only a few bacterial counts were carried out on B. There was a large degree of fluctuation in the antibiotic (B1) set ; biomass values increased to 47,22 up from 8,9 $pg.dm^{-3}$. The majority of bacterial biomass values was found to lie between 5 and 25 $pg.dm^{-3}$. Control AODC values were found in a range of 15 to 22 $pg.dm^{-3}$, with the exception of the 10 min. reading of 53 $pg.dm^{-3}$. The 180 min. reading was found to be 4,47.

3.3.4.2. Tritiated thymidine activity study.

Tables 6 and 7 show the amounts of residual activity in the filtrate expressed as disintegrations per minute (DPM).

The DPM values were obtained from scintillation counter readings derived from subsampling intervals (indicated by a,b,c,d,e and f). These subsamples originated from a sample taken at each one of the normal exudation intervals (0, 5, 10 mins.) and subjected to the incubation procedure.

BACTERIAL BIOMASS TABLE

A1	0	5	10	15	30	60	90	120	150	180	210	240
A1	7,37	12,31	-	25,94	29,62	5,41	21,60	15,97	14,05	13,67	16,02	18,99
A2	12,19	29,90	10,18	25,67	18,08	16,02	29,81	34,90	4,95	-	16,78	6,28
A3	20,42	34,75	25,75	136,01	41,26	22,49	31,42	28,11	43,22	30,49	-	36,56
B1	-	-	8,94	47,22	6,28	13,25	16,35	-	20,91	12,04	19,12	-
B2	-	-	-	-	-	12,76	-	-	22,01	-	-	-
C	18,56	17,70	53,52	16,29	6,29	20,58	17,44	16,88	12,02	4,47	20,86	21,17

TABLE 5 : Bacterial biomass values expressed in picogram (bact. biomass).dm^{-3}

No	Sampling interval	a	b	c	d	e	f	
1	0	0	48	119	179	239	303	sl = 5,502
		2043	2136	2545	2674	2934	3891	r^2= 0,943
2	5	5	65	128	185	248	308	sl = 11,99
		655	2304	2694	3211	3920	4683	r^2= 0,976
3	10							
4	15	11	71	139	199	259	318	sl = 6,495
		2189	2092	2919	3503	-	3931	r^2= 0,959
5	30	29	89	152	239	273	333	sl = 14,807
		1028	2539	2837	3168	5088	6022	r^2= 0,946
6	60	59	119	179	239	303	363	sl = 11,344
		787	2921	2591	3644	-	4662	r^2= 0,925
7	90	89	152	239	273	333	393	sl = 13,71
		958	1539	2504	3356	4011	5113	r^2= 0,992
8	120	119	179	239	303	363	423	sl = 8,072
		1027	1300	1729	3698	2918	3096	r^2= 0,874
9	150	152	239	273	333	393	453	sl = 8,937
		1136	2386	2294	3969	3287	3895	r^2= 0,895
10	180							
11	180	179	239	303	363	423	-	sl = 11,509
		607	1079	1966	2723	3303		r^2= 0,997
12	210	214	273	333	393	453	-	sl = 6,67
		1181	1250	1838	2428	2581		r^2= 0,972
13	240	239	303	393	423	453	-	sl = 0,12
		1451	5225	2331	2551	2835		r^2= -0,01

TABLE 6 : Exp. 3 Thymidine incubation study on experimental container A1.

Second column = Sampling times at which the incubation was initiated.
Columns a-f = Subsampling times / DPM scintillation counting data pairs :
In any row, the upper figure denotes the time (in mins.) when subsampling took place ; the lower figure indicating the corresponding DPM count.
Last column = regression parameters :
sl = slope of line, r2 = correlation coefficient.

No	Sampling interval	a	b	c	d	e	f	
1	0	0	48	119	179	239	303	sl = 17.807
		911	1559	2438	4016	3991	6769	r^2= 0,964
2	5	5	65	128	185	248	308	sl = 15,229
		1500	1589	3347	3649	5232	5705	r^2= 0,979
4	15	11	71	139	199	258	318	sl = 12,263
		1506	1578	2596	3665	-	4992	r^2= 0,984
5	30	29	89	152	239	273	333	sl = 16,635
		1402	2388	3820	3485	5951	6757	r^2= 0,940
6	60	59	119	179	239	303	363	sl = 17,288
		575	3086	3011	4256	-	6322	r^2= 0,965
7	90	89	152	239	273	333	393	sl = 22,992
		1219	2961	3545	4644	9372	6953	r^2= 0,878
8	120	119	179	239	303	363	423	sl = -2,516
		2065	1544	1188	1396	1010	1272	r^2= 0,781
9	150	152	239	273	333	393	453	sl = 1,68
		940	827	806	1281	1314	1257	r^2= 0,766
11	180	179	239	303	363	423	-	sl = -1,828
		1166	699	786	739	585		r^2=-0,802
12	210	214	273	333	393	453	-	sl = -0,268
		1011	735	800	931	832		r^2=-0,232
13	240	239	303	393	423	453	-	sl =-3,125
		551	4894	1198	2215	961		r^2=-0,159

TABLE 7 : Exp. 3 ^{3}H-Thymidine study on control container; legend as in table 6.

The first column displays a number linking the uptake sequence to a linear uptake curve. At the end of the row, data pertaining to a linear regression are listed. The experimental set data of thymidine uptake is represented graphically in Fig. 12. As can be seen from the regression coefficients, uptake of thymidine by bacteria can well be described by a linear regression with r-values closely approaching 1.

The slopes of the graphs of the experiment were found to be positive to varying degrees. Only the last graph (13 at 240 mins.) did not follow a linear trend (Table 6).

In the control there was a tendency from uniformly steep gradients (rapid uptake and high activity) to less rapid uptake and lower activity . The slopes of the graphs in the latter half of the experiment were approaching zero or negative values (which were taken as equal to zero since negative uptake values are meaningless). In the experiment the decrease in activity was reversed and delayed for 1 hour after the kelp had been removed from the system.

Plotting bacterial biomass (as numbers) and activity vs. time, activity was observed to reflect biomass trends (Fig. 12a). Upon the introduction of the kelp into the system, there were 2 pulses of activity : at 5 and 15 mins. These were followed directly by concomitant increases in biomass (comparing Figs. 4 and 12a, see Appendix 3). After 15 mins. there was a decrease until 60 mins. (note : slopes of lines equal). The slopes of both lines were found to be similar after 120 min. when the kelp was taken out.

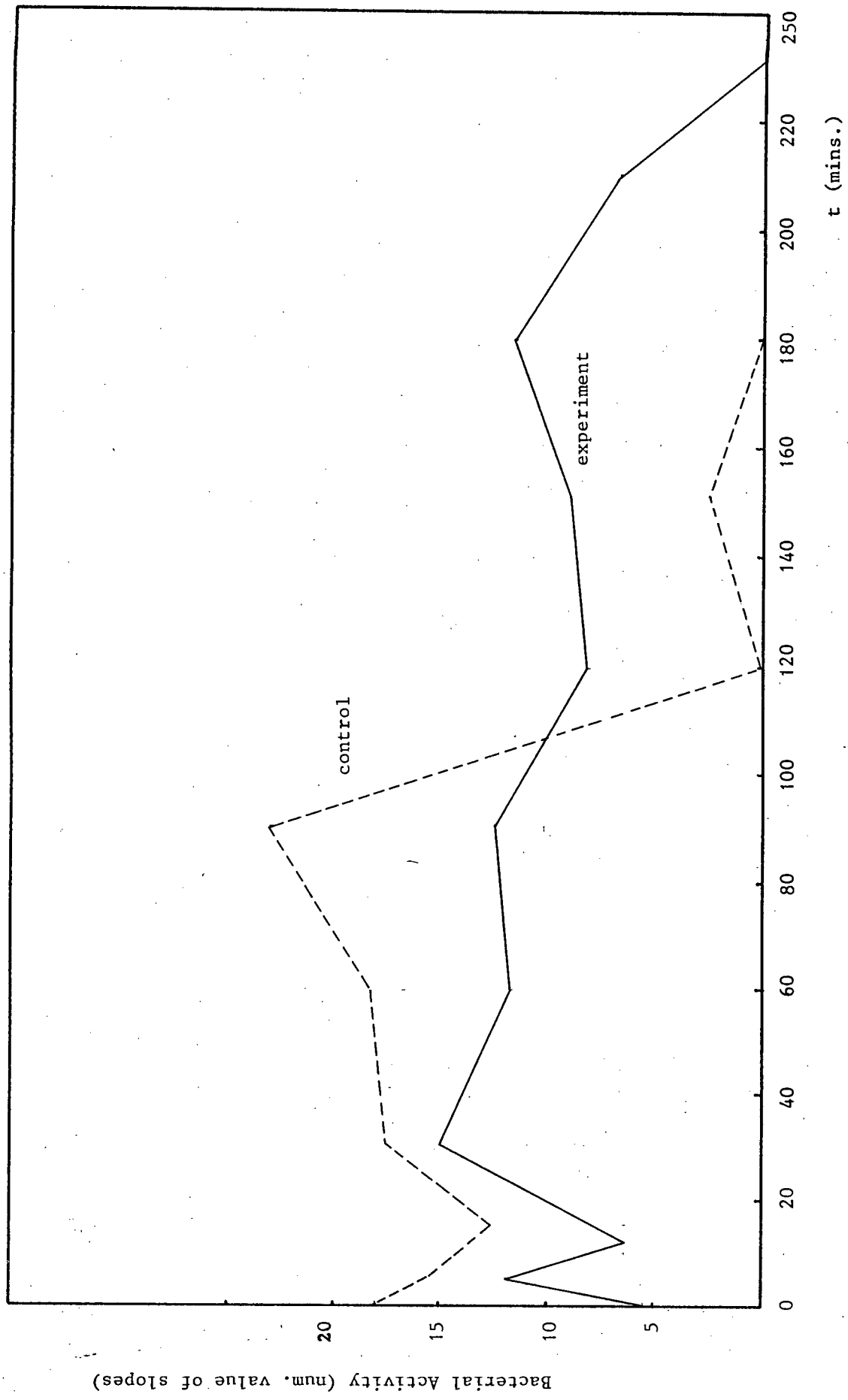


Fig. 12 : Monitoring of bacterial activity (thymidine incubation) in A1

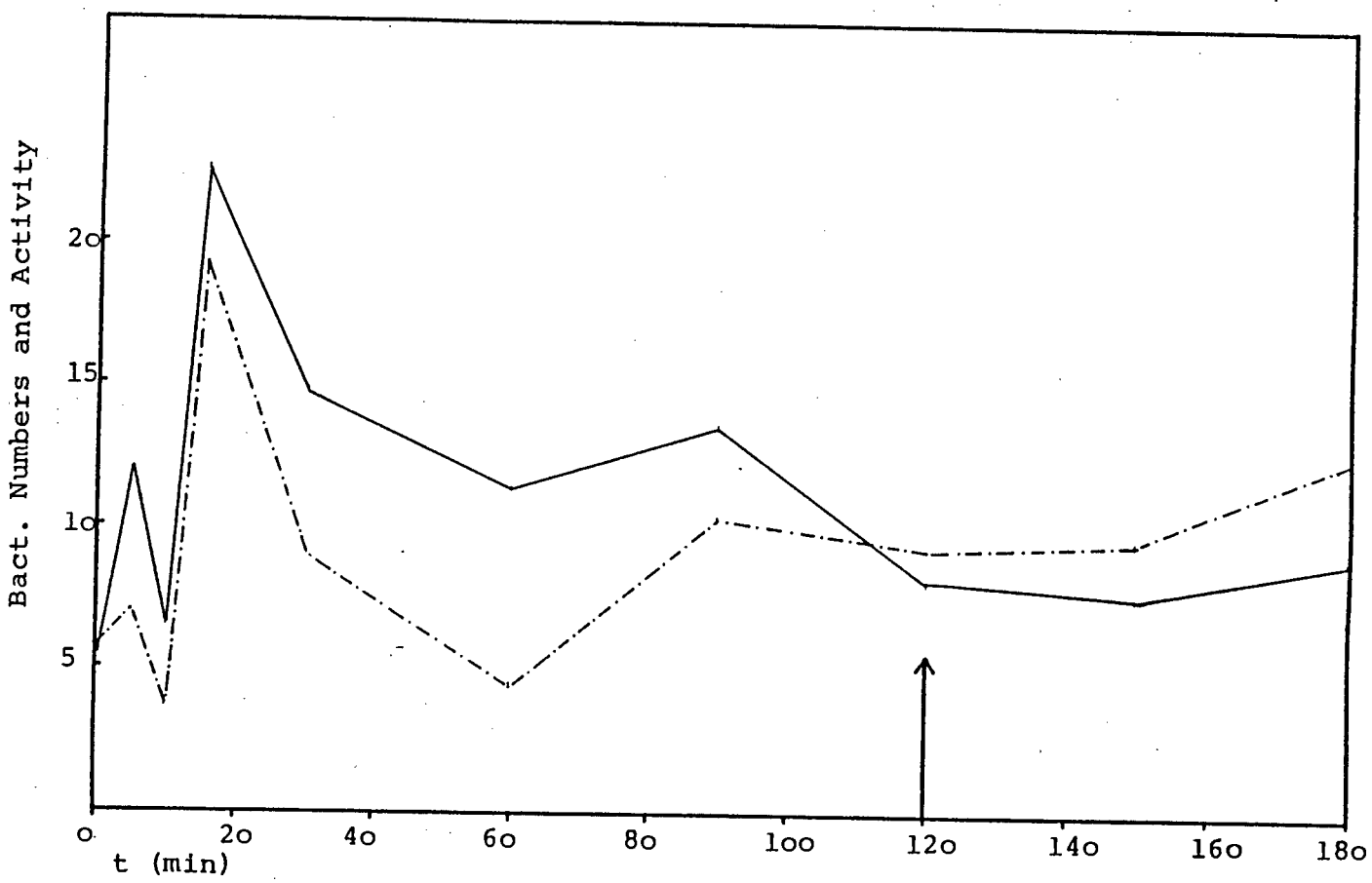


Fig. 12a : Comparison between bacterial numbers (broken line) and bacterial activity (unbroken line), in A1.

y-axis equal for both :
Bact. numbers : No. per microscope field (direct)
Bact. activity : DPM scintillation count.

3.4. DISCUSSION

3.4.1. DOM Release

The question arises as to whether the results of the DOM studies reflect an experimental artifact. The determination of the total N levels has shown similarity in pattern of all three replicates of experiment 3 (Fig. 4 & 5). Common to all 3 sets was an initial pulse of DON release during the first 15 minutes of the experiment, similar to the one that could be observed in the pilot studies. Initial pulsing of DOM was noted by Carlson & Carlson (1984) in discrete collections of exudate. The kelp presumably reacts to the disturbance - in the form of a change in habitat - by stress-provoked extracellular release (Moebus, et al, 1974). The fact that the DON that is traced during this initial pulse disappeared as quickly as it was formed would suggest a highly efficient removal system. In this case, the removal of metabolites could be initiated by a combination of any of the following three factors :

Seaweeds are known to utilize organically bound nitrogen, although at lower rates than inorganic nitrogen (deBoer, et al, 1978). In order to minimise the loss of DON, the kelp may therefore actively reabsorb part of its previously liberated organically bound N. Secondly, the phytoplankton present in the system (unfiltered seawater) may be responsible for uptake and release of organic N (Wheeler & Hellebust, 1981), especially since the presence of a major producer of photosynthates such as Ecklonia maxima could have a beneficial effect on the growth of coastal phytoplankton. Thirdly, bacteria and, to a much lesser extent flagellates, form the bulk of the heterotrophic micro-organisms present in the system (Linley & Newell, 1981). The observed peaks of DON can be hypothesized to represent pulses of

exudation occurring at irregular intervals. Alternatively, a lack of DON increase (as between 15 and 30 mins. in all replicates) could indicate an efficient bacterial removal system operating simultaneously with extracellular release. This would account for the lack of net accumulation of DON but does not exclude the possibility of a relatively high flux, ie. DOM changing pools within the system.

Data obtained for the release of DON therefore do not reflect release exclusively : rather, they are a result of various processes that are taking place concurrently within the system. Adding an antibiotic solution to the system was assumed to only affect prokaryotic heterotrophs by preventing cell division. It was found that release values for the treated experiments were low, often being below the level of the control. No change occurred in SE values after the kelp had been taken out (Appendix 2). This would indicate that no liberation of organically bound N had taken place that could be utilized by micro-organisms. The antibiotics possibly not only affected the bacterial flora but also epidermal cells of the thallus, effectively preventing exudation from taking place. This is indirect evidence that exudation occurs as an active energy-dependent process at the interface of thallus and ambient seawater.

Most of the total N recorded is made up of two components : nitrate and DON. Ammonium levels were consistently low at around 1-1,5 $\mu mol.dm^{-3}$. Nitrate levels were higher and as such influence the total N much more than ammonium (Appendix 2). Nitrate values were below 10 $\mu mol.dm^{-3}$ clustering at around 5 $\mu mol.dm^{-3}$ which is low for South Atlantic inshore waters (Chapman & Shannon, 1985). Similar to the fluctuations observed in the DON levels, DIN was in a state of flux as a result of

removal and denitrification processes occurring simultaneously with production and exudation. Furthermore, the finding that ammonia was present at only low and natural levels would indicate the absence of anoxia or other degradation processes.

Total N and DON levels in experiment 6 were found to be higher than in 3. There was very little actual accumulation of DON. The gradual increase in DON between 120 and 250 mins. corresponded to a net production of 2,27 $\mu mol.dm^{-3}.hr^{-1}$. Including the initial release between 0 and 5 mins., the total amount of kelp-derived DON was 4,88 $\mu mol.dm^{-3}.hr^{-1}$ or, on a mass basis, 353,8 $\mu mol\ N.(kg\ dwt)^{-1}.hr^{-1}$.

Under natural conditions, the initial pulse release might not occur and so the rate of DON release could be tentatively determined as 164,6 $\mu mol\ N.(kg\ dwt)^{-1}.hr^{-1}$. This figure would reflect the exudation rate of E. maxima during winter (June) and is based only on the visible phases of accumulation. This figure could thus be an underestimation, but is supported by the estimation of DON release from the kelp during August : 65,92 $\mu mol\ N.(kg\ dwt)^{-1}.hr^{-1}$ are released between 5 and 120 mins. in the study (see Fig. 7).

The results for the discrete collections were higher because the initial pulse of exudate was replicated and determined the exudation rate of DON. Changing the water every 15 mins. had the effect of diminishing heterotrophic activity and utilisation of the algally derived substrate as well as possible metabolite resorption. From the continuous collection results it could be seen that after the initial pulse a rapid decrease in organic matter levels followed immediately, and often no new organic input could be monitored thereafter.

Rates of release derived from discrete collections can therefore be considered as follows : Either, i) They represent an

artificial system, whereby release is prompted by continual contact with water relatively devoid of organics as well as having a low bacterial population count. Or, ii) They may constitute a true representation of an open flow-through system as is present in the natural environment.

Results obtained as an average of initial 15 mins exudation pulses tend to show a constant high level production of dissolved organics by the thallus. Calculating release rates from the data presented in Fig. 8 results in a pooled estimate of DON release in all 7 sets monitored. A figure of 138,23 $\mu mol.dm^{-3}$ was obtained, which is equivalent to 13,35 mmol $N.(kg\ dwt)^{-1}.hr^{-1}$.

Similarly, kelp A in experiment 5 (Fig. 9) was calculated to have an exudation rate of 7,498 mmol $N.(kg\ dwt)^{-1}.hr^{-1}$. The rate for Bwas similar to that of A : 7,84 mmol $N.(kg\ dwt)^{-1}.hr^{-1}$. It becomes apparent that the rate obtained in March was higher than in May again possibly because of seasonality effects. Due to the difference in magnitude between the rates of release obtained during continuous collections and those derived from discrete collections, no direct comparison is possible.

A similar trend was visible when considering DOC release in June as compared to the August measurement. Chemical oxygen demand (COD) in the June experiment (6) can be described as analogous to the DON determinations of the October experiment (3), because of i) the characteristic initial pulse (14 $mg.l^{-1}.O_2$) and rapid disappearance, ii) the decreasing COD values between 15 and 60 mins. and iii) the peak that appeared at 60 mins. had shifted to the 90 min. mark. DOC release is estimated to have taken place at a rate of 12,96 $mg.l^{-1}.O_2$. Compared to this rate of release, COD was calculated as only 5 $mg.l^{-1}.O_2$ (ignoring the initial pulse) during the experiment in late August (8).

3.4.2. Heterotrophic Utilization

The results of the thymidine incubation showed that the experimental container received an input of substrate that was bacterially utilized. It is significant that substrate uptake took place even though the kelp had been removed after 120 mins as seen by the positive slopes of the graphs. The control was characterised by more initial uniformity lacking the metabolite input from the kelp. As a result, the slopes in the latter half of the experiment were approaching zero or even negative values, indicating that the nutrient reserve in the form of DOM had become limiting to the growth and activity of micro-heterotrophs. The control was seen to exhibit a higher bacterial activity with less fluctuations than in the experiment, although it followed the same trends. After 90 mins. the control system "crashed" and hardly any bacterial activity (ie. multiplying populations) was detected thereafter. This contrasts with the experiment where a decrease in activity was reversed and delayed until 1 hour after the kelp had been removed from the system.

The bacterial activity and biomass measurements can be used to explain variations in the concentrations of DOM. Comparing the combined activity / bacterial numbers graph in Fig. 12a with the release of DON (Fig. 4), the pronounced pulse of DON released during exudation at 10 mins. into the experiment was followed directly afterwards by a steep increase in biomass and bacterial activity ; the bacteria readily utilising the DON as a substrate (Appendix 3). Figs. 4 and 12a Similarly, the DON release peak at 60 mins. in A1 was utilised immediately ; as seen by the increase in activity between 60 and 90 mins. and the simultaneous increase in biomass. When the kelp was taken out, the substrate input was eliminated but the residual DOM was sufficient to allow the

bacteria to thrive even after the source of substrate was removed. The system can therefore be described as follows : Organic matter with a high C:N ratio enters the system as a result of macroalgal exudation (Newell & Field, 1983) and undergoes relative enrichment (decrease in the C:N ratio) upon the rapid utilization of that DOM (Reichardt & Dieckmann, 1985) leading to the regeneration of N.

3.4.3. DFAA Results

In all the DFAA studies the possibility of obtaining artifacts must be considered. These can arise as a result of shearing of bacterial and phytoplankton cells upon vacuum filtration. Filter pore size and type have been found to affect amino acid profiles: Nuclepore and GF/F Whatman filters apparently induce DFAA release from marine micro-organisms (Fuhrman & Bell, 1985). These filters were used in this study as well.

DFAA are a form of DOM and their concentrations in seawater represent a balance between release processes (producers) and removal (heterotrophs). i) The characteristic release peaks were observed during the first 15 minutes in experiment 2 and 4b, similar to the initial peaks of DON (section 3.4.1.). ii) Concentrations of DFAA were higher towards the end of the experiments indicating DON accumulation as a result of extracellular release. iii) An increase in DFAA quantities was found to cause an increase in the number of individual amino acids measured.

Amino acids in seawater are considered to be a good substrate for marine heterotrophs providing a carbon- as well as a nitrogen source. The action of heterotrophs explains the presence of a threshold level of DFAA concentrations as postulated by Lee &

Bada (1977). DFAA levels in coastal seawater have been found to vary between 0,2-0,6 $\mu mol.l^{-1}$ (Garrasi, et al, 1979) and 1-2,4 $\mu mol.l^{-1}$ (Clark, et al, 1972). Compared to this range of accepted DFAA concentrations, the values found in this study were measured to be higher (between 1,98 and 8,3 $\mu mol.l^{-1}$ obtained after 24 hours). The latter value, however, does not represent exudation exclusively ; cell lysis and excessive bacterial colonization may have been responsible for the high DFAA level.

It has been known for a long time that alanine, and to a lesser extent, aspartic acid and glutamine are prominent in a variety of macroalgae (Channing & Young, 1953). Coastal seawater reflects this pattern because of the abundance of these amino acids in primary producers (Clark, et al, 1972). The occurrence of glutamic acid has been interpreted in terms of the importance of the glutamine / glutamate pathway of nitrogen assimilation (Syrett, 1981). In this study, glutamic acid was not present before the kelp was introduced (exp. 4b), but detected at a concentration of 0,04 to 0,12 $\mu mol.l^{-1}$ later and thus appears to be kelp-derived. Glutamine was not detected ; it may be that it is selectively retained by the plant during conditions of abundant nitrogen supply to facilitate the assimilation of that nitrogen (Miflin & Lea, 1977). Arginine, alanine, cysteine, lysine and tyrosine were detected in the pre-experimental blank. It is assumed that coastal seawater naturally contains these amino acids because other DFAA are either selectively removed by heterotrophs or do not enter the water in detectable quantities. Amano, et al, (1982) have shown that DFAA utilization activity of marine bacteria determines the DFAA composition, depending on the particular proteolytic strain of bacteria present.

C H A P T E R

I V

NUTRIENT UPTAKE AND ASSIMILATION STUDIES ON *Ecklonia maxima*

4.1. INTRODUCTION

4.1.1. The Uptake of Nitrogen

Nitrogen is regarded as the most commonly limiting major nutrient in coastal waters to the growth of marine macrophytes (Harrison & Druehl, 1981). Mainly because of their size, little work has been done on the nitrogen nutrition of whole thalli of the larger seaweeds, concentrating instead on experiments using disks cut from blades (Wheeler, 1982).

Several internal and external environmental factors can influence nutrient uptake by healthy thalli. Light affects nutrient uptake in a number of ways : i) in providing the energy for active uptake and subsequent transport processes, ii) to produce carbon skeletons as sinks for incorporated ions, iii) to stimulate growth which results in an increased demand for mineral nutrients (Lobban, et al, 1985). The rate of diffusion, and cell metabolism in general, is directly dependent on temperature. Two other factors influence rates of uptake : Firstly, some ions can competitively interact with the uptake of other ions, especially when they have the same ionic charge. Secondly, water motion is very important in dispelling depleted concentration gradients around the thallus. This explains why seaweeds may grow in turbulent but nutrient deficient environments as often observed along rocky shores (deBoer, 1981).

Although preferable, experimentation under field conditions has a number of disadvantages. Firstly, natural conditions, such as insolation and variation in ambient nutrient level in the sea, must be taken into account. Secondly, due to the nature of simulated field experiments, there are practical difficulties in producing a statistically viable number of replicates. Thirdly, laboratory studies are more successful in terms of precision and

sampling, not the least because the experiments are often scaled down (Harrison & Druehl, 1981). Data obtained under laboratory conditions can be extrapolated to describe natural conditions only tentatively ; the corollary being that field experiments reflect natural processes more accurately, though the results may be rather more difficult to interpret. Therefore the results obtained during the course of these uptake studies - and indeed, of all those in chapter 3,4, and 5 - should be seen in the light of these limitations.

The uptake of inorganic forms of nitrogen exhibits saturation kinetics in most seaweeds (Hanisak & Harlin, 1978). The uptake of NH_4 in Codium fragile subsp. tomentosoides increased with increasing ammonium concentrations up to a certain point (5uM), beyond which little or no increase was observed (Hanisak & Harlin, 1978). Similarly, NO_3 uptake did not parallel external concentrations beyond a specific higher external concentration in Laminaria longicruris : Harlin & Craigie (1978) reported a maximum uptake rate (Vmax) of nitrate to range from 7 to 10 $\mu mol.(g\ dwt)^{-1}.hr^{-1}$. In their study, ammonium uptake did not affect nitrate uptake. This Vmax figure contrasts with the one determined for Macrocystis pyrifera (L.) C.Ag. (Haines & Wheeler, 1978) : The Vmax here was 30,5 $\mu mol.(g\ dwt)^{-1}.hr^{-1}$. To explain their high uptake figures, these authors presented evidence suggesting a second uptake mechanism for Macrocystis. Vmax figures in Fucus spiralis L. were found to be similar for nitrate, ammonium, as well as nitrite suggesting common uptake systems at the plasmalemma. On a relative basis, nitrate, ammonium and nitrite were estimated to contribute 59, 39 and 2%, respectively, to the yearly N uptake by the apical frond of Fucus spiralis (Topinka, 1978).

Using a flow-through system, Probyn & Chapman (1982) investigated the nitrogen uptake characteristics of a brown alga, Chordaria flagelliformis. (O.F.Müll.) C.Ag. NH4 uptake was studied from two angles : as a batch mode (incorporating N-enrichment) and an apparent steady state system (continuous mode with a continually sufficient N supply). The maximum uptake rates (Vmax) measured in short term depletion experiments greatly exceeded those determined under a steady nutrient supply. This obviously has implications regarding the estimation of actual uptake rates based on depletion-of-nutrient experiments.

Of particular relevance to this work is the study by Probyn & McQuaid (1985) on the in situ measurement of N-uptake substances in Ecklonia maxima. Using plastic bag enclosures to monitor the depletion of nitrogenous nutrients, the following main points emerged as a result of their study : E. maxima took up nitrate and ammonium from the ambient seawater, but not urea and showed only a weak preference for ammonia. Nitrate uptake was linearly related to the external concentration of nitrate, indicating that the tissue contents of nitrate (or its assimilated form) would be higher under conditions of upwelling. Further, the uptake of nitrate did not saturate at levels >20 $\mu mol.dm^{-3}$. Compared to similar nitrogen levels experienced by Macrocystis, E. maxima had an uptake rate of nitrogen that is about four times lower, reflecting the higher growth potential that has been reported for Macrocystis pyrifera (Gerard, 1982). During upwelling, rates of N-uptake by E. maxima are high ; it is possible that nitrogen is stored for times of seasonally low ambient N levels.

4.1.2. Aims

The aim of this section was to study nutrient uptake (with

emphasis on nitrogen) in E. maxima by measuring concentrations of nitrate, ammonium and phosphate in the surrounding seawater. Uptake velocities were determined for ambient and enriched substrate concentrations at different times of the year. Tissue amino acids (non-protein) were quantified to detect assimilation patterns of nitrogen into the macroalgal thallus.

4.2. MATERIALS AND METHODS

Nutrient concentrations (nitrate, ammonium, phosphate and silica) were determined in duplicate according to the descriptions in section 2.6.

Non-protein free amino acid (FAA) concentrations were determined in ethanol tissue extracts that were prepared during some experiments. This was done according to section 2.7.5.2.
Sampling for tissue analysis was done at the start of the experiment, after 30 mins. and after 60 mins (only in exp. 7). Results were compared against a no-nitrogen blank obtained from a control experiment conducted concurrently.

4.3. RESULTS

4.3.1. Uptake of added Ammonium Nitrate (NH_4NO_3)

As can be seen in Fig. 13, ammonium was rapidly taken up. From an initial value of 81,6 µmol $N.dm^{-3}$, ammonium levels reached almost total depletion (0,4 µmol.dm^{-3}) within 185 mins. Nitrate was taken up by the kelp much more slowly : From 108,35 µmol $N.dm^{-3}$ at the beginning to 95,63 µmol $.dm^{-3}$ at the end of the experimental period. While almost 100% of the total available ammonium (TAA) was taken up, only 11,7% of the total available

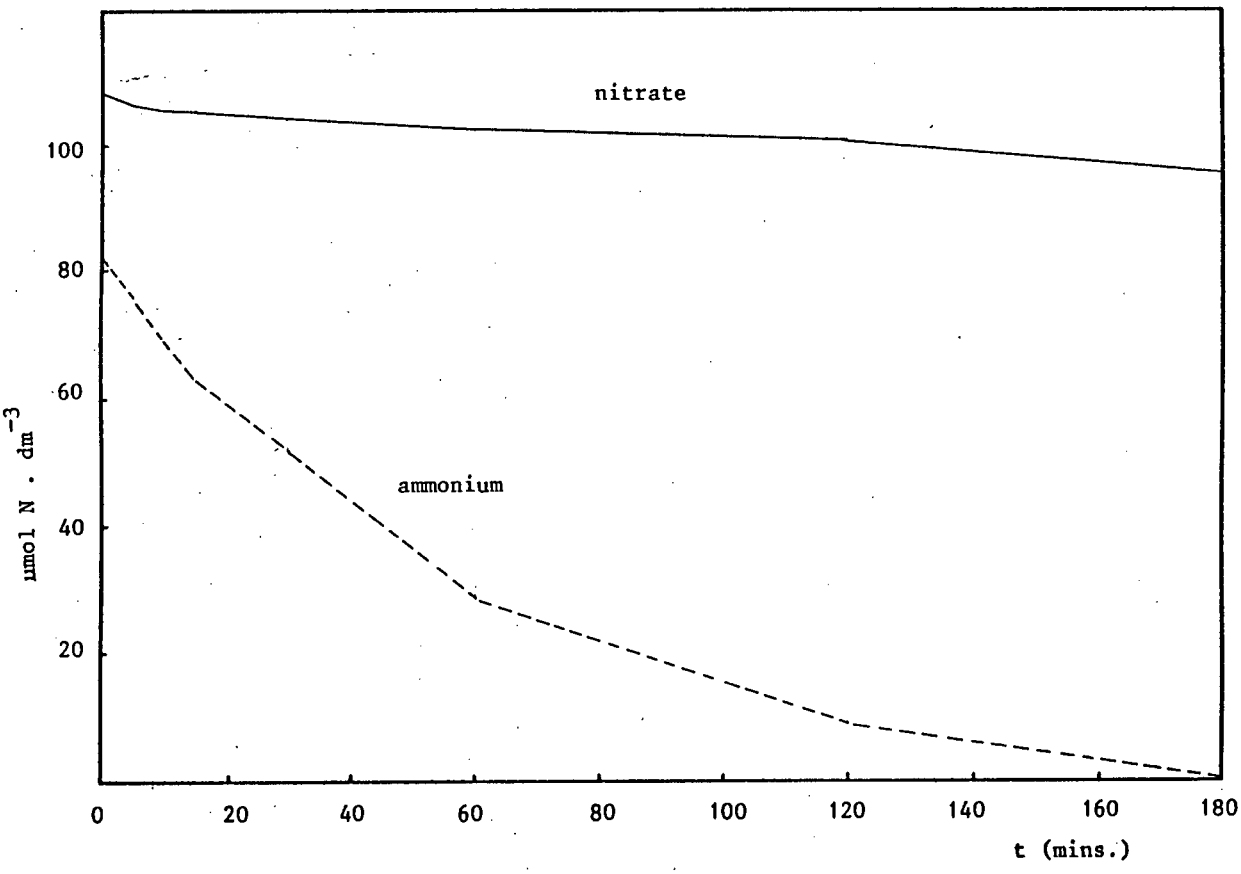


Fig. 13 : Ammonium nitrate uptake experiment (4b)

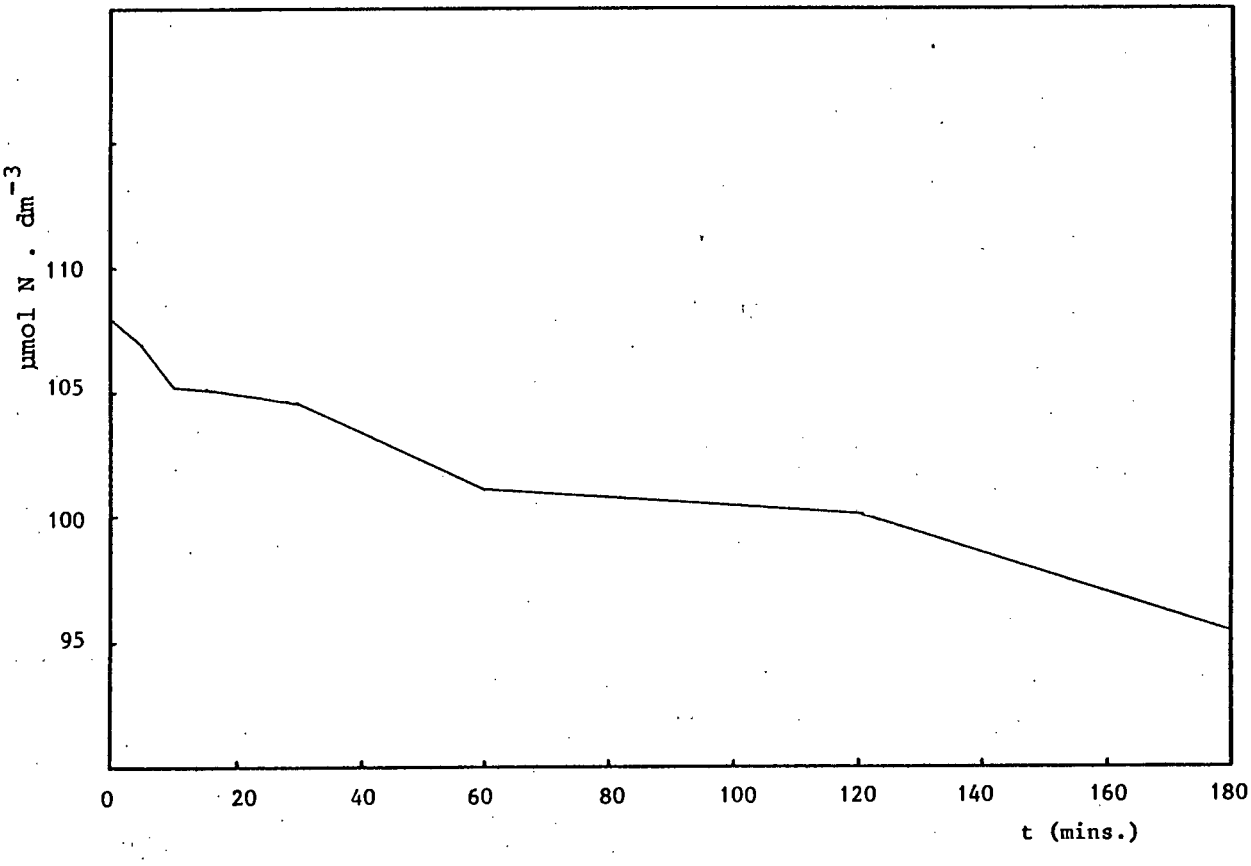


Fig. 14 : Exp. 4b ; Nitrate uptake curve

nitrate (TAN) was taken up in approximately 3 hours. Nitrate uptake occurred in a linear fashion and can be described by the following equation :

$y = -0,00593x + 106,56$ ($r^2 = -0,97$). However, Fig. 14 shows that rapid uptake took place during the first 10 mins. and can be described as $y = -0,31x + 108,23$ ($r^2 = -0,99$). The remainder of the nitrate depletion curve conforms to

$y = -0,0537x + 105,853$ ($r^2 = -0,99$).

Ammonium uptake fits an exponential regression curve, where $y = 103,21\ e^{(-0,0027x)}$ ($r^2 = -0,94$). Uptake was linear and rapid during the first 15 mins. when almost 23% of the TAA had been taken up. This initial phase of uptake can be described by

$y = -1,236x + 81,82$ ($r^2 = -0,99$) and the second part as

$y = -0,32x + 54,26$ ($r^2 = -0,95$).

The N-uptake rates can be summarised as follows :

NO_3 :

At [S] = 108,35 $\mu mol.dm^{-3}$:

852,6 µg TAN taken up (= 5,62% of TAN) per hour

$V = 8,24$ mg $(kg\ dwt)^{-1}.hr^{-1}$, or

$= 589\ \mu mol\ (kg\ dwt)^{-1}.hr^{-1}$

NH_4 :

At [S] = 81,6 $\mu mol.dm^{-3}$:

7280 µg TAA taken up (= 63,73% of TAA) per hour

$V = 70,34$ mg $(kg\ dwt)^{-1}.hr^{-1}$, or

$= 5020\ \mu mol\ (kg\ dwt)^{-1}.hr^{-1}$

4.3.2. Enrichment of Seawater with Additional Nitrate

Fig. 15 shows the uptake of nitrate, ammonium and phosphate when nitrate was present in enriched quantities.

Nitrate at a concentration of 44,78 µmol $N.dm^{-3}$ was taken up rapidly within 5 mins. reaching a concentration of 40 $umol.dm^{-3}$ where it was found to remain constant for another 10 mins. At the end of the experiment, nitrate was present at 33,52 $\mu mol.dm^{-3}$ Disregarding the initial rapid absorption of NO_3, the following regression line can be fitted (from 15 to 185 mins.) :

$y = - 0,042x + 40,51$ ($r^2 = -0,97$).

Ammonium was present in the system at low levels (t=0 ; 2,22 $umol.dm^{-3}$) and, except for the first 10 mins., no clearly defined uptake of ammonium was found to have occurred. Rather, ammonium concentrations fluctuated and increase slightly in concentration towards the end of the experiment (3,72 $\mu mol.dm^{-3}$). The uptake of inorganic phosphorus at natural concentrations (t=0; 1,51 µmol $P.dm^{-3}$) was also investigated. Uptake of total available phosphorus (TAP) occurred between 5 and 15 mins. (steep decline) and again between 30 and 90 mins. (shallow), which made calculation of uptake rates meaningless.

Data are summarised as follows :

At enriched nitrate levels ;

NO_3 :

At [S] = 44,78 $\mu mol.dm^{-3}$:

1112,16 µg TAN taken up (= 14,78% of TAN) per hour

$V = 5,248$ mg $(kg\ dwt)^{-1}.hr^{-1}$, or

$= 374,76$ µmol $(kg\ dwt)^{-1}.hr^{-1}$

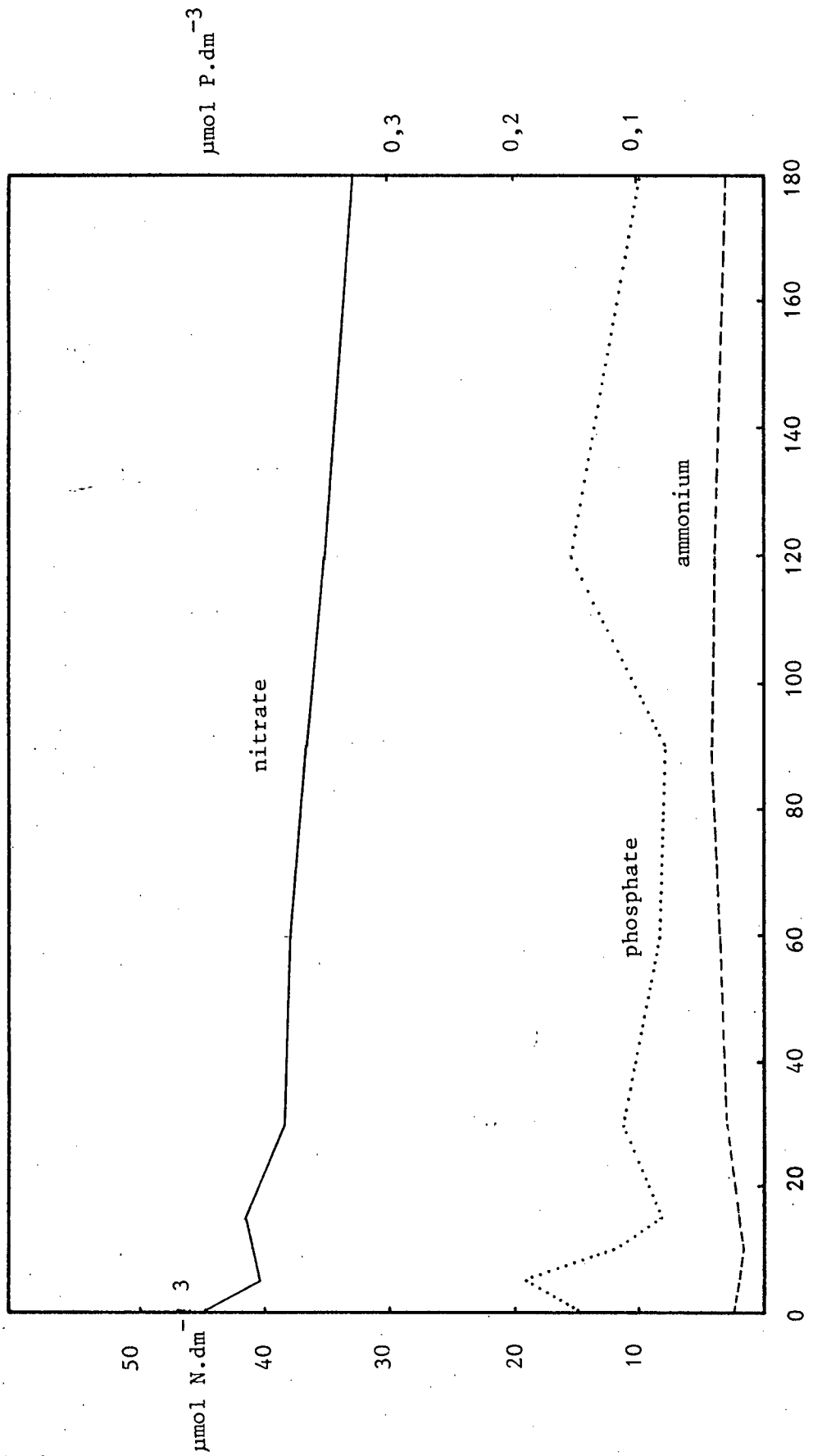


Fig. 15 : Exp. 7 ; Monitoring of nitrate, ammonium & phosphate at enriched nitrate levels

4.3.3. Enrichment of Seawater with Additional Ammonium

Fig. 16 shows the uptake of nitrate, ammonium and phosphate under enriched ammonium conditions. Ammonium was taken up rapidly within the first 30 mins. (29,43 to 6,8 $\mu mol.dm^{-3}$). Uptake then slowed down, and a final value of 2,11 $\mu mol.dm^{-3}$ was obtained at the end of the experiment. The uptake kinetics can be described by 2 separate linear regressions :

(0-30 mins.) : $y = -0,72x + 26,5$ ($r^2 = -0,96$)

(30-180 ") : $y = -0,03x + 7,66$ ($r^2 = -0,97$)

Nitrate uptake followed a gradual dissappearance of the medium from an initial concentration of 13,23 $\mu mol.dm^{-3}$ of which 9,45 $\mu mol.dm^{-3}$ remained at the end of the experiment. Nitrate uptake (Fig. 16) could be described as :

$y = -0,02x + 12,93$ ($r^2 = -0,99$)

Phosphate uptake was found to be similar to the ammonium uptake because of the initially fast removal of substrate. From a concentration of 1,63 $\mu mol.dm^{-3}$, the kelp was found to rapidly remove about half of the TAP within the first 10 mins. (0,78 $\mu mol\ P.dm^{-3}$) ; thereafter P-uptake was much more gradual. Final value of P after 3 hours was 0,3 $\mu mol\ P.dm^{-3}$.

Summarising the results of experiment 7 ;

At enriched ammonium levels :

NH_4 :

At [S] = 29,43 $\mu mol.dm^{-3}$:

3932,4 µg TAA taken up (= 79,5% of TAA) per hour

$V = 18,44\ mg\ (kg\ dwt)^{-1}.hr^{-1}$, or

$= 1320\ \mu mol\ (kg\ dwt)^{-1}.hr^{-1}$

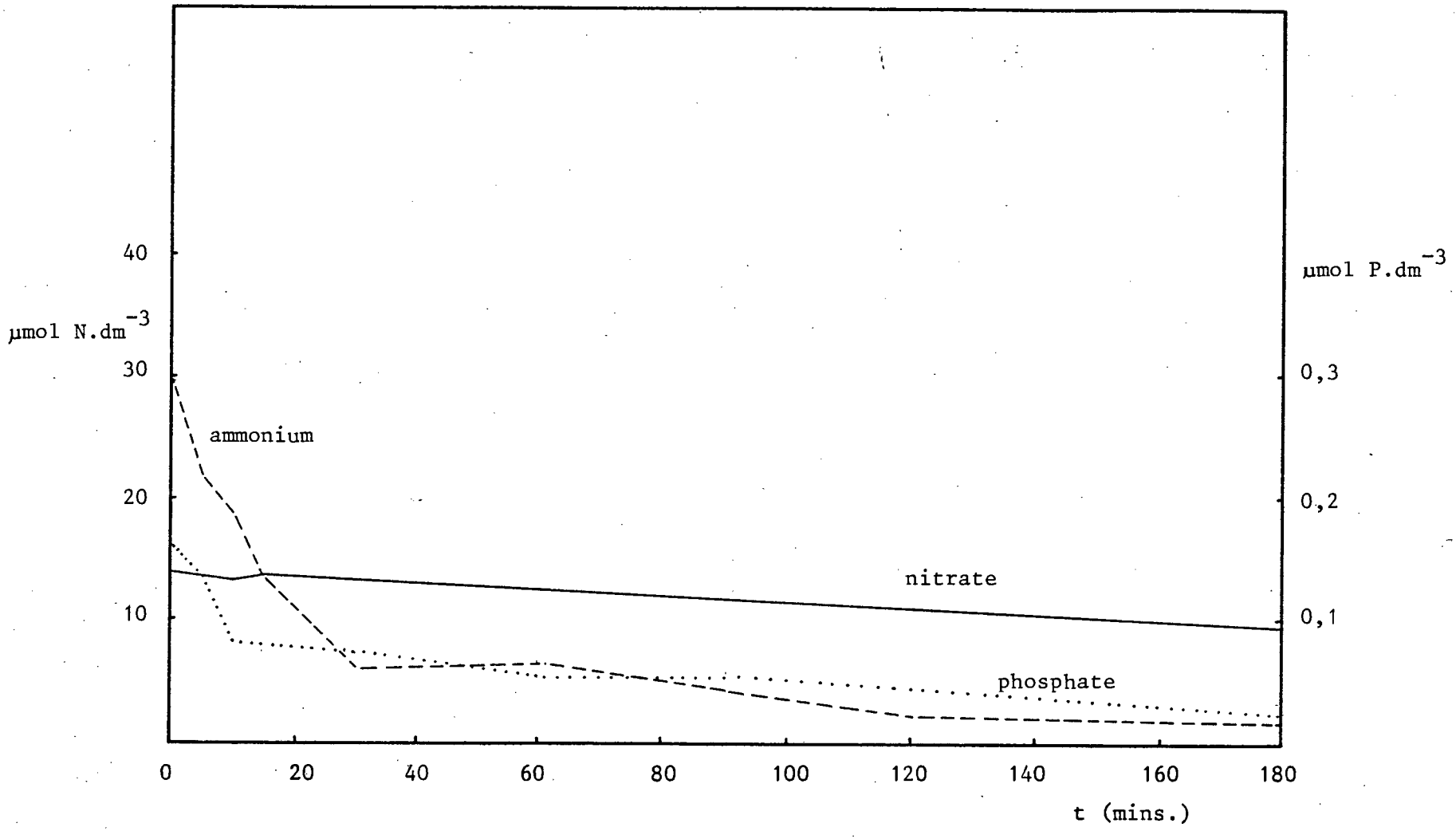


Fig. 16 : Exp. 7 ; Monitoring of nitrate, ammonium & phosphate at enriched ammonium levels

NO_3 :

At [S] = 13,23 $\mu mol.dm^{-3}$:

294 µg TAN taken up (= 13,2% of TAN) per hour

V = 1,38 mg $(kg\ dwt)^{-1}.hr^{-1}$, or

= 98,56 µmol $(kg\ dwt)^{-1}.hr^{-1}$

P :

At [S] = 1,63 $\mu mol.dm^{-3}$:

405,12 µg TAP taken up (= 68,88% of TAP) per hour

V = 1,89 mg $(kg\ dwt)^{-1}.hr^{-1}$

= 61,22 µmol $(kg\ dwt)^{-1}.hr^{-1}$

4.3.4. Uptake at Ambient Nutrient Levels

During the discrete collection experiment (4a), nitrate uptake at ambient levels took place repeatedly (Fig. 17). The concentration of nitrate in the seawater was determined to be 11,35 $umol.dm^{-3}$. After 15 mins. the level of nitrate in the buckets had decreased to between 10,5 to 10,6 $\mu mol.dm^{-3}$ indicating uptake. The first 5 sets showed the same type of uptake curve, especially after the first 5 mins. No uptake was found to occur between 10 and 15 mins. in set 6. The following uptake regression was calculated :

$y = -0,051\ (+-0,004)x + 11,284(+-0,071)\quad (r^2 = 0,95\ +-0,0042)$

Because of the naturally low concentrations of ammonium in seawater, fluctuations and error margins were disproportionately large (Fig. 18). After the first 2 ammonia monitored sets which show these fluctuations the successive sets show rapid uptake of ammonium during the first 5 mins., but none or only very little thereafter (eg. 4 and 6). Uptake of ammonium was calculated using an average derived from sets 3 to 7.

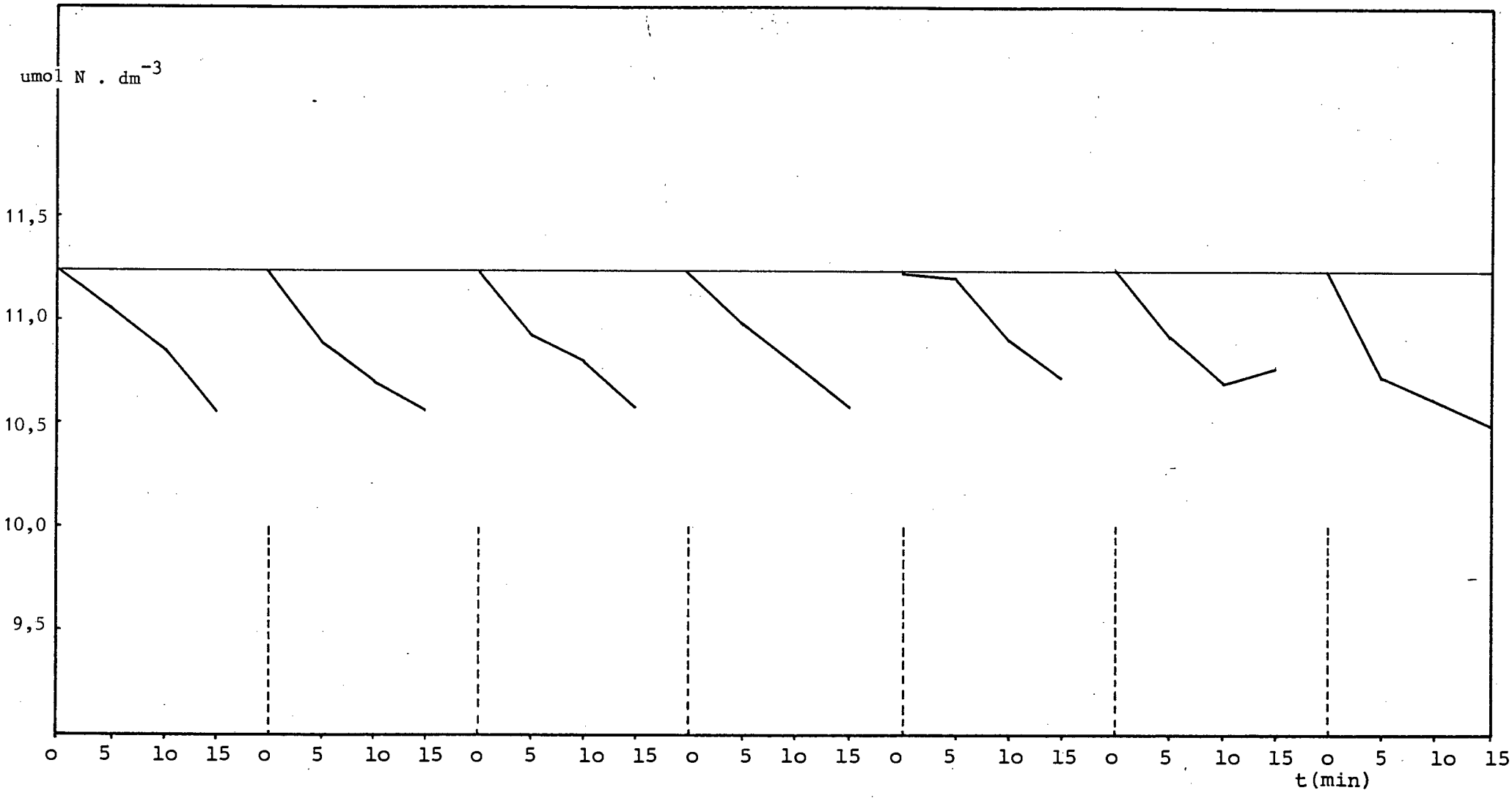


Fig. 17 : Exp. 4a ; Monitoring of nitrate concentrations (discrete coll.)

Background concentration indicated by line.

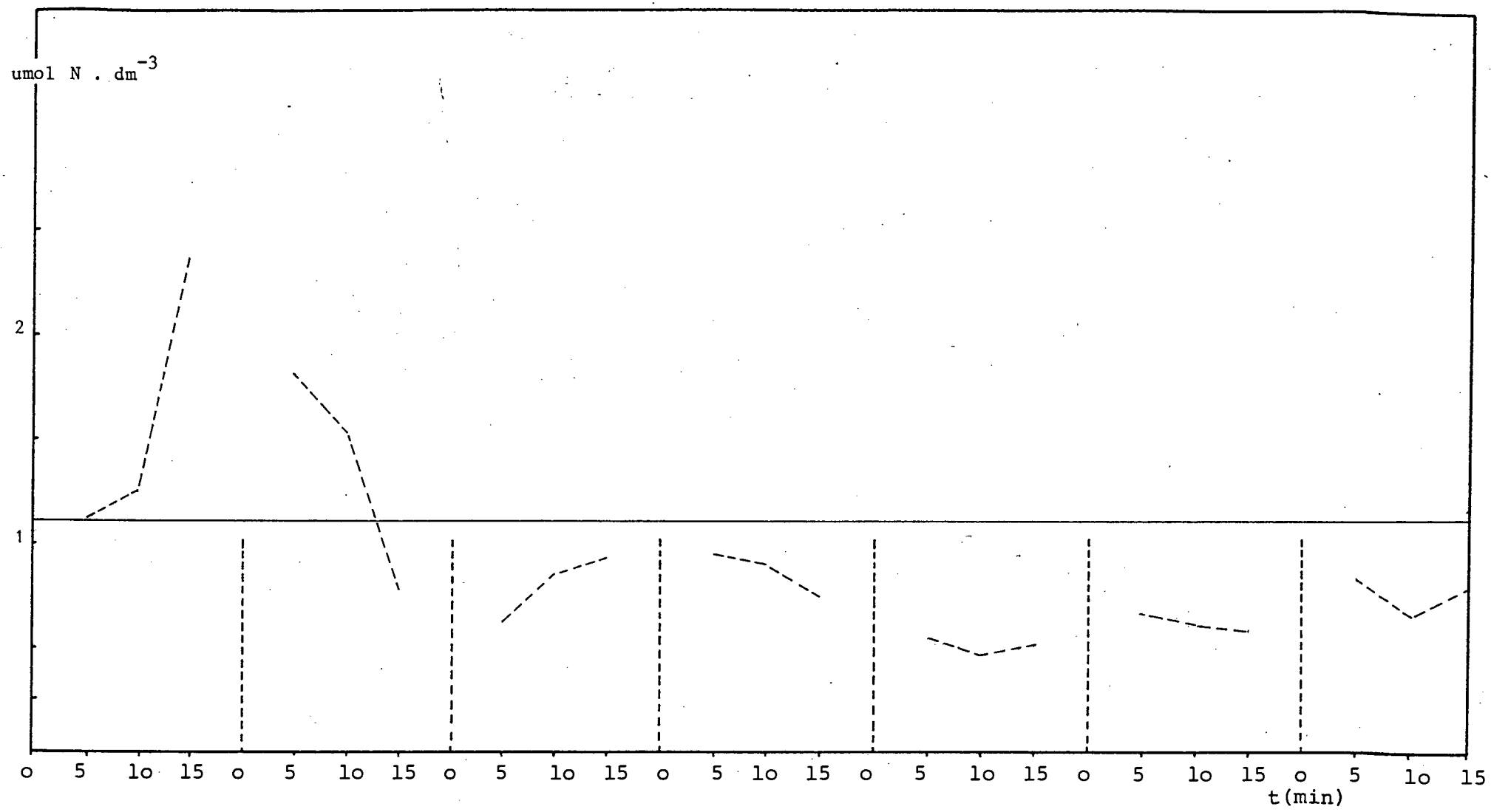


Fig. 18 : Exp. 4a ; Monitoring of ammonium concentrations (discrete coll.)

Background concentration indicated by line.

At ambient DIN levels :

NO_3 :

At [S] = 11,35 µmol.dm^{-3} :

424,8 µg TAN taken up (= 6,69% of TAN) per hour

V = 4,185 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 299 µmol (kg dwt)$^{-1}$.hr^{-1}

NH_4 :

At [S] = 1,2 µmol.dm^{-3} :

296,8 mg TAA taken up (= 44,17% of TAA) per hour

V = 2,924 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 208,8 µmol (kg dwt)$^{-1}$.hr^{-1}

The data of experiment 5 demonstrated a higher amount of variability between replicas and discrete sets. The ambient nitrate concentration was determined at 12,18 µmol.dm^{-3} (Fig. 19). The following uptake rates were calculated for nitrate by determining the initial and final (15 min.) concentrations of the nutrient (in Appendix 2) :

NO_3 (A) :

At [S] = 12,18 µmol.dm^{-3} :

406,4 µg TAN taken up (= 5,96% of TAN) per hour

V = 1,595 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 113,9 µmol (kg dwt)$^{-1}$.hr^{-1}

NO_3 (B) :

At [S] = 12,17 µmol.dm^{-3} :

261,4 µg TAN taken up (= 3,83% of TAN) per hour

V = 1,331 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 95,1 µmol (kg dwt)$^{-1}$.hr^{-1}

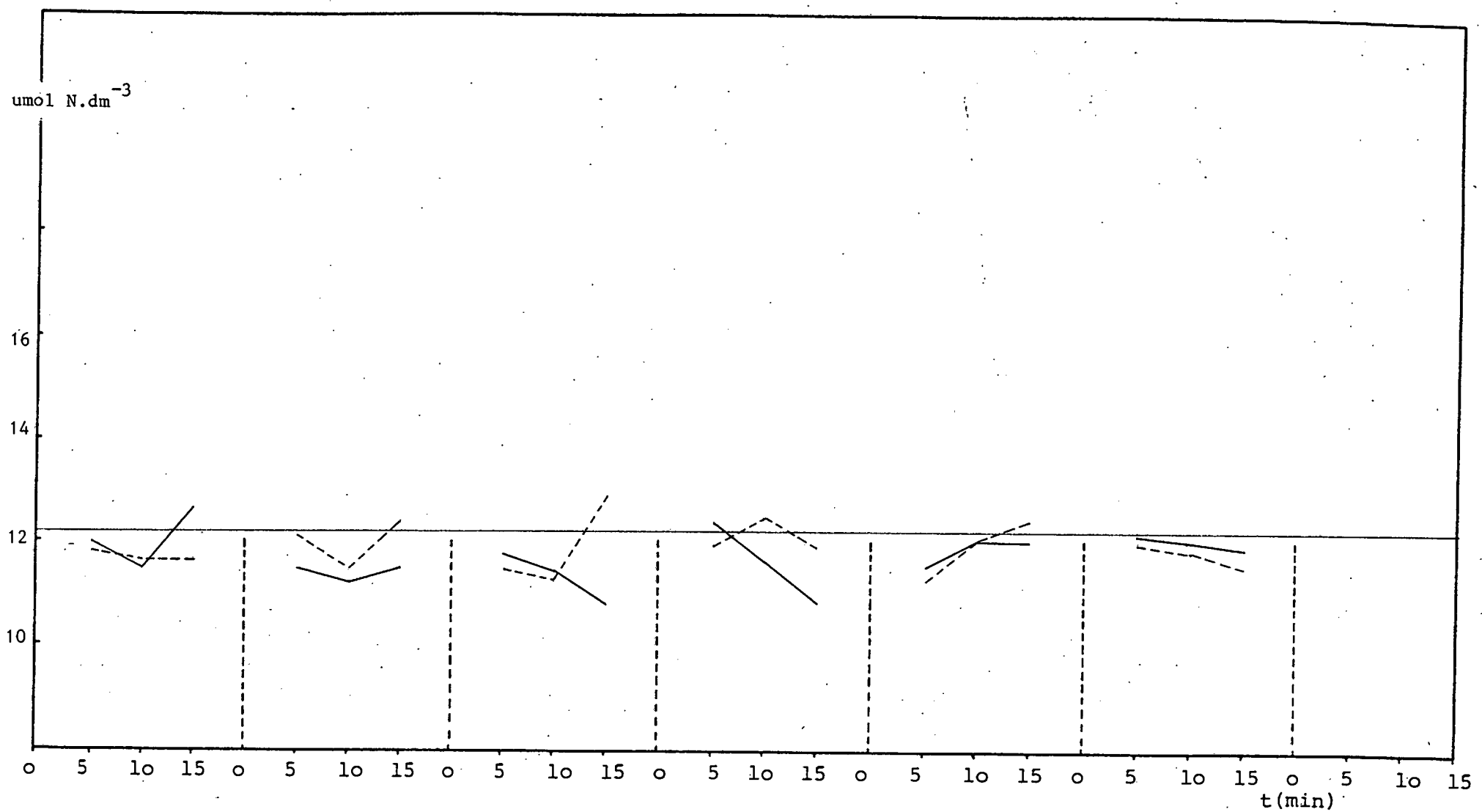


Fig. 19 : Exp. 5 ; Monitoring of nitrate concentrations (discrete coll.)

Background concentration indicated by line.

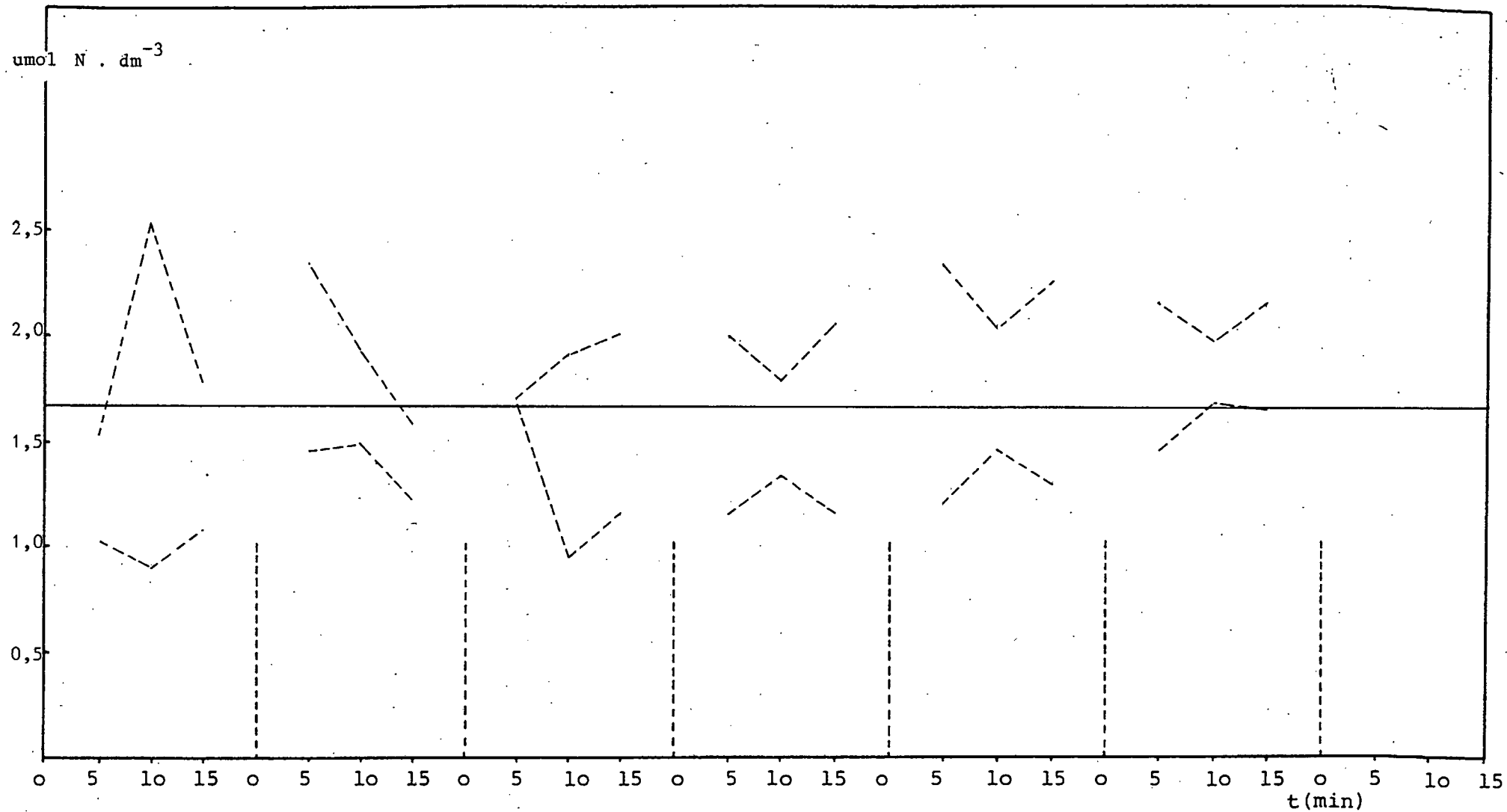


Fig. 20 : Exp. 5 ; Monitoring of ammonium concentrations (discrete coll.)

Background concentration indicated by line.

Fig. 20 indicates that ammonium uptake was very irregular in this experiment and non-reproducible throughout its duration. Uptake rates could only tentatively be determined for kelp A using an average of sets 2, 3, 4 and 5. Ambient ammonium levels were at 1,64 $\mu mol.dm^{-3}$. There was very little uptake and therefore difficult to quantify :

NH_4 :

At [S] = 1,64 $\mu mol.dm^{-3}$:

240,8 µg TAA taken up (= 26,22% TAA) per hour

V = 0,945 mg $(kg\ dwt)^{-1}.hr^{-1}$, or

= 67,5 µmol $(kg\ dwt)^{-1}.hr^{-1}$

The monitoring of phosphate and silica levels is shown in Fig. 21. The ambient P level was analysed as 0,925 µmol $P.dm^{-3}$. Monitoring the dissappearance of P in the medium showed a slight uptake of P to have taken place. Within 15 mins, the concentration of phosphate had decreased from 0,925 to 0,85 $\mu mol.dm^{-3}$ in the medium. Using pooled data the following uptake rates can be calculated :

P (A):

At [S] = 0,925 $\mu mol.dm^{-3}$:

127,6 µg TAP taken up (= 11,13% TAP) per hour

V = 0,501 mg $(kg\ dwt)^{-1}.hr^{-1}$, or

= 16,2 µmol $(kg\ dwt)^{-1}.hr^{-1}$

P (B):

At [S] = 0,925 $\mu mol.dm^{-3}$:

55,6 µg TAP taken up (= 4,86% TAP) per hour

V = 0,283 mg $(kg\ dwt)^{-1}.hr^{-1}$, or

= 9,14 µmol $(kg\ dwt)^{-1}.hr^{-1}$

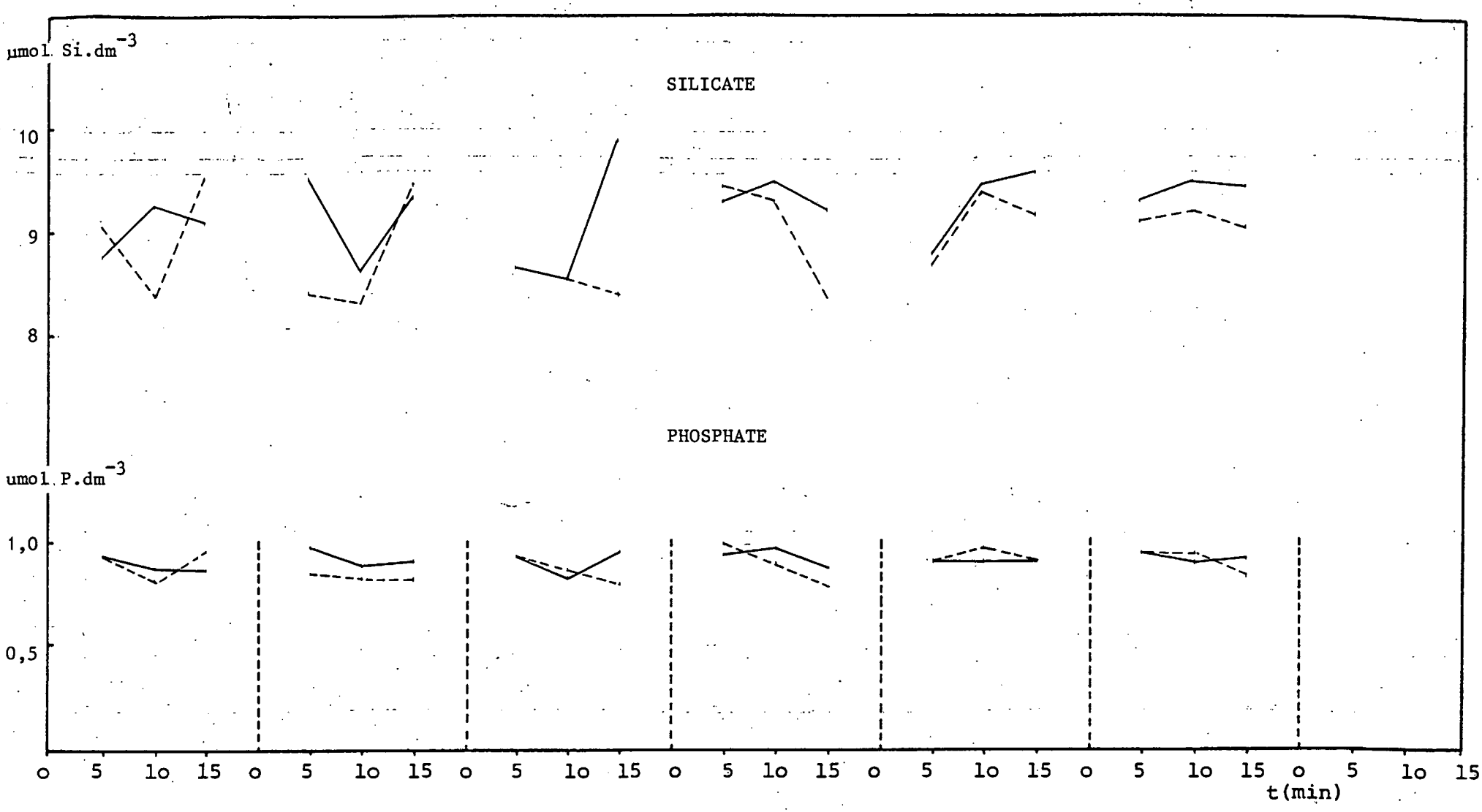


Fig. 21 : Exp. 5 ; Monitoring of silicate and phosphate (discrete coll.)

There were no real trends visible when considering the level of silica in the experimental buckets. The fluctuations of Si did not follow a general direction and were found to be centred around a background seawater concentration of 9,065 µmol Si.dm^{-3}. Minimum to maximum concentration values ranged from 8,34 to 9,9 µmol Si.dm^{-3}.

Fig. 22 shows the dissappearance of nitrate from the medium in continuous collection experiment 6. From an initial value of 17,46 umol.dm^{-3}, nitrate concentration decreased to 14,29 µmol.dm^{-3} within 4 hours, 10 mins. Initial fluctuations between 0 and 15 mins were of a minor magnitude. Nitrate uptake in this case can be described by :

$y = -0,013x + 17,53$ $(r^2 = -0,996)$

Except for the first 15-30 mins. of the experiment, no depletion of ammonium ions in the water could be detected and hence no uptake (Fig. 23). Ammonium concentrations were found to be lower at the beginning than those obtained after 250 mins. ; 1,43 and 1,7 µmol.dm^{-3}, respectively. The quality of the data did not allow for the calculation of uptake parameters.

There was no change in silica level throughout the duration of experiment 6. Initial levels were similar to final levels (Fig. 25 in Appendix 3).

Phosphate was present in the seawater at 1,08 µmol.dm^{-3} and was taken up by the kelp linearly until a final concentration of 0,78 µmol P.dm^{-3} was reached at the end (Experiment 6, Fig. 24) :

The regression curve for P-uptake was :

$y = -1,77x10^{-3}x + 1,127$ $(r^2 = -0,93)$

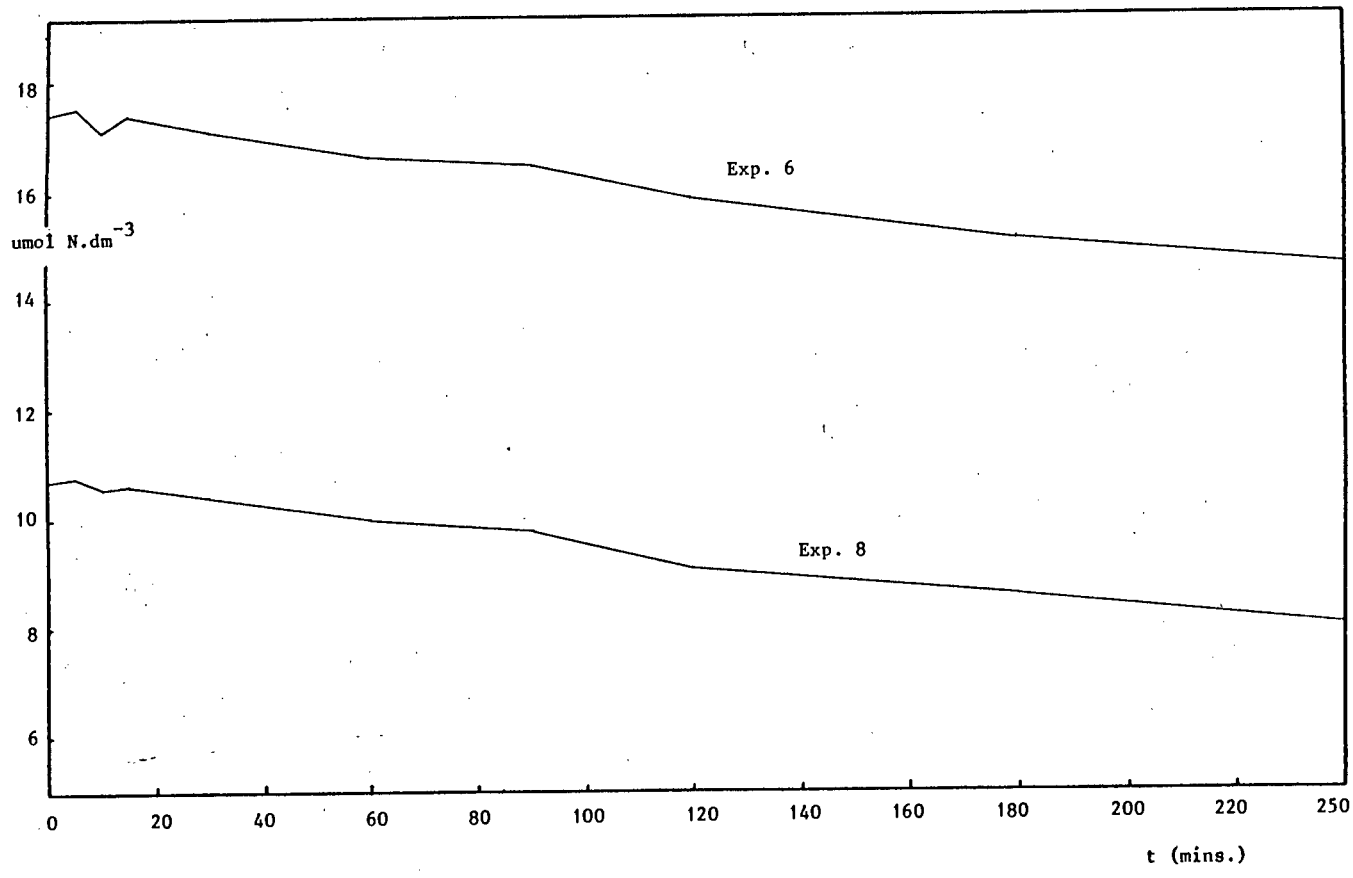


Fig. 22 : Exp. 6 & 8 ; Monitoring of nitrate concentrations

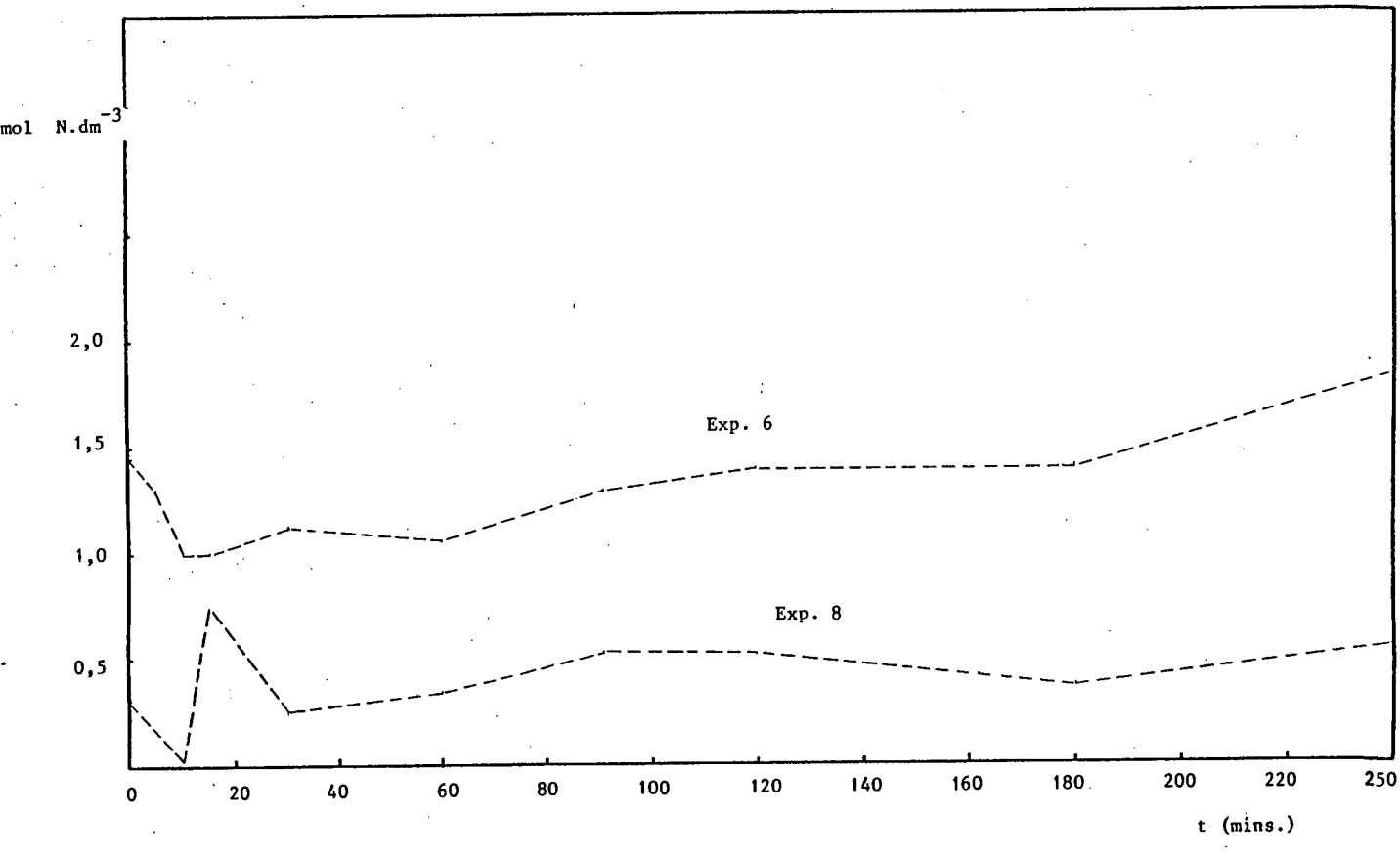


Fig. 23 : Exp. 6 & 8 ; Monitoring of ammonium concentrations

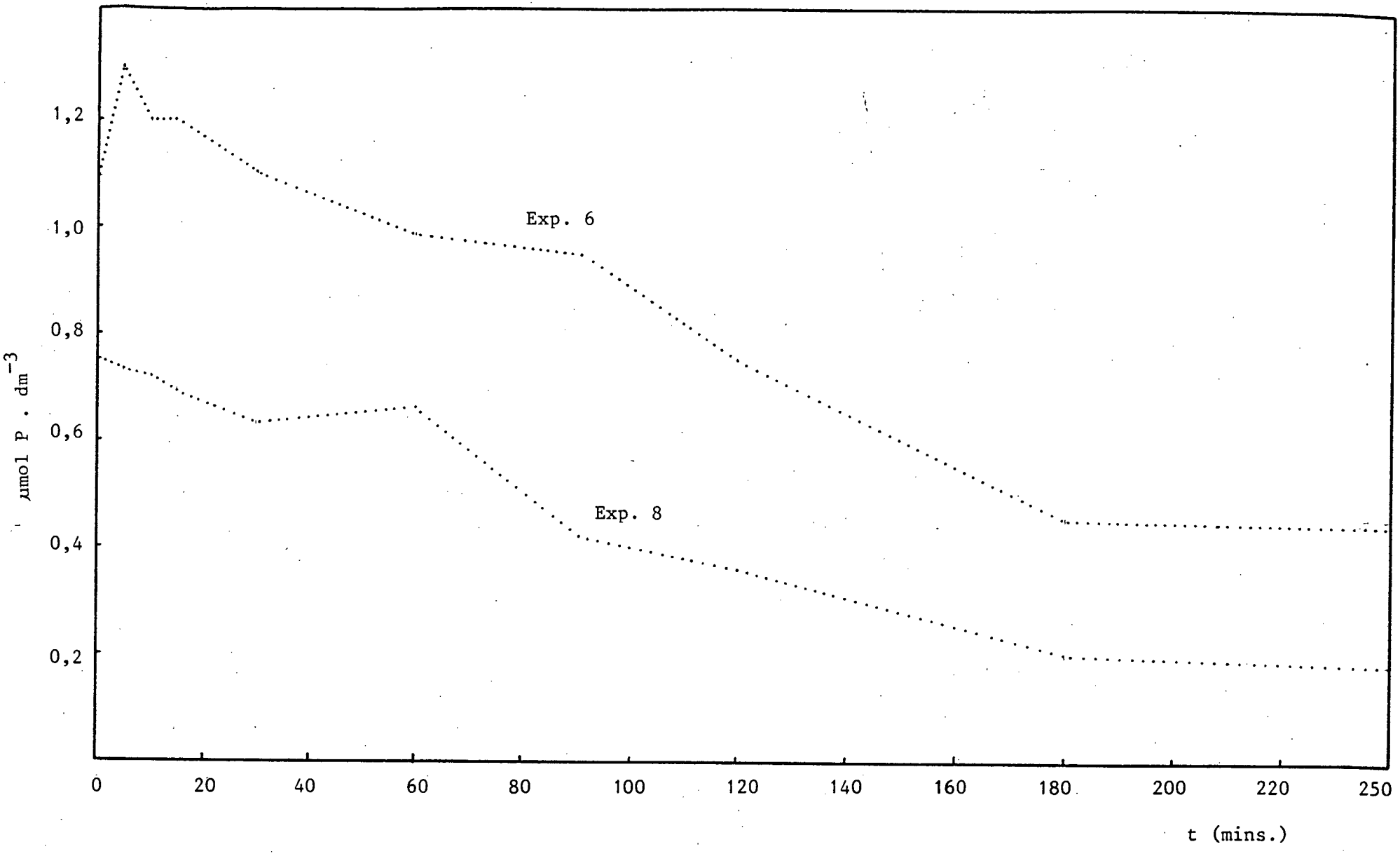


Fig. 24 : Exp. 6 & 8 ; Monitoring of phosphate concentrations

NO_3 :

At [S] = 17,46 µmol.dm^{-3} :

159,75 µg TAN taken up (= 4,36% TAN) per hour

V = 0,772 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 55,15 µmol (kg dwt)$^{-1}$.hr^{-1}

P :

At [S] = 1,08 µmol.dm^{-3} :

4,95 µg TAP taken up (=13,89% TAP) per hour

V = 0,359 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 11,6 µmol (kg dwt)$^{-1}$.hr^{-1}

Uptake of nitrate and phosphate was evident during experiment 8. Nitrate followed linear uptake kinetics with initial levels at 10,7 and final concentrations at 8 µmol.dm^{-3} :

$y = -0,011x + 10,69$ ($r^2 = -0,99$) (see Fig. 22).

Fig. 21 shows that P was taken up by the kelp. Initial concentration was 0,75 µmol P.dm^{-3} decreasing to 0,19 µmol.dm^{-3} after 260 mins. Uptake also followed linear kinetics :

$y = -2,44x10^{-3} + 0,723$ ($r^2 = -0,95$)

NO_3 :

At [S] = 10,70 µmol.dm^{-3} :

10,08 µg TAN taken up (= 6,73% TAN) per hour

V = 0,544 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 38,8 µmol (kg dwt)$^{-1}$.hr^{-1}

P :

At [S] = 0,75 µmol.dm^{-3} :

2,48 µg TAP taken up (= 10,67% TAP) per hour

V = 0,134 mg (kg dwt)$^{-1}$.hr^{-1}, or

= 4,31 µmol (kg dwt)$^{-1}$.hr^{-1}

4.3.5. Levels of Non-Protein Free Amino Acids (FAA) in Tissue Samples of Ecklonia maxima

The results obtained for the amino acid analysis of the ammonium-nitrate enrichment experiment (exp. 4b) are given in Table 8 and can be summarised as follows :

a) all amino acids increased in concentration between 0 and 30 mins.

b) the most important amino acids in descending order (with percentage increase in brackets) were :

alanine	(34,6%)
glutamine	(10,8%)
glutamic acid	(27,4%)
aspartic acid	(24,2%)
serine	(20,3%)
arginine	(44,9%)
threonine	(10,1%)

c) proline, valine, lysine and histidine were present only in traces amounts.

Table 9 presents the results of the amino acid analyses of the experiment 7 (section 4.2.). Trends can be summarised as follows:

a) Alanine, glutamic acid, aspartic acid and glutamine were the most common FAA with initial (No-N) values of 4,763; 2,658; 2,374 and 1,737 µmol (g fwt)$^{-1}$, respectively.

b) There was a slight increase in the amount of FAA during the first 30 mins. of nitrate feeding (97,44 - 93,28 µmol nitrate.dm^{-3}) as expressed by the major FAA and the FAA total (13,4 vs. 14,5 µmol (g fwt)$^{-1}$. However, there was a decrease after 30 mins. as shown by the 60 min. analysis. All the FAA decreased in concentration as compared to the initial analysis. The nitrate level had reached 88,64 µmol.dm^{-3} after 60 mins. ; 91% of the original concentration. Tissue ammonia had increased, however.

	init.	3o mins.	init.	3o mins
Amino Acid	µmol g^{-1}(dw)-DFAA		%N	
aspartic acid	3.oo4	3.965	.816	.417
threonine	o.664	o.739	.o78	.o868
serine	o.8o3	1.oo8	.1o7	.134
asparagine	o.349	o.336	.o651	.o627
glutamic acid	3.o74	4.233	.293	.4o3
glutamine	6.533	7.324	1.25	1.4
proline		trace		
glycine	o.341	o.4o3	.o586	.o752
alanine	2o.o52	3o.642	3.15	4.81
valine		trace		
isoleucine	trace	o.134	trace	.o143
leucine	nil	o.134	nil	.o143
tyrosine	trace	o.2o2	trace	.o156
phenylalanine	o.349	o.47o	.o296	.o399
lysine		trace		
histidine		trace		
arginine	o.629	1.142	.2o2	.367
γ- amino butyric	o.175	1.142	.o238	.155
NH_3 / NH_4^+	8.21	8.534	(6.57	6.83)
TOTAL (-NH)	35.95	52.479	5.66	8.17

TABLE 8 : FAA in Kelp Tissue during Enrichment with Ammonium-Nitrate (Exp. 4b)

Initial $[NO_3]$ = 108 µM N

Final $[NO_3]$ = 102 µM N

Initial $[NH_4]$ = 82 µM N

Final $[NH_4]$ = 30 µM N

AMINO ACID	No-N	NH_4 30	NH_4 60	NO_3 30	NO_3 60
Aspartic acid	2,374	1,889	2,577	2,544	1,988
Threonine	0,134	0,11	0,141	0,097	0,104
Serine	0,253	0,16	0,236	0,263	0,233
Asparagine	0,169	0,093	0,133	0,164	0,159
Glutamic acid	2,658	1,723	2,084	2,569	2,187
Glutamine	1,737	1,703	2,027	1,751	1,626
Glycine	0,062	0,068	0,068	0,079	0,038
Alanine	4,763	3,401	4,349	5,065	4,252
Valine	0,051	0,04	0,051	0,025	0,025
Cystine	0,042	0,026	0,047	0,037	0,04
Isoleucine	0,101	0,063	0,097	0,048	0,069
Leucine	0,033	0,019	0,015	0,016	0,021
Tyrosine	0,049	0,069	0,078	0,034	0,045
Phenylalanine	0,036	0,044	0,044	0,049	0,045
Ammonia	0,766	0,862	1,162	1,462	0,929
Lysine	0,149	0,178	0,071	0,274	0,073
Arginine	0,056	0,033	0,077	-	-
Ethanolamine	-	0,14	0,178	-	-
	13,433	10,648	13,435	14,477	11,834

Table 9 : Kelp FAA During Nitrogen Feeding Experiment 7 (Aug).
Values in µmol (g fwt)$^{-1}$.

Initial $[NO_3]$ = 97 uM N

Final $[NO_3]$ = 88 uM N

Initial $[NH_4]$ = 37 uM N

Final $[NH_4]$ = 30 uM N

c) The kelp fed with ammonium (initial level = 37,31 µmol.dm^{-3}) exhibited a decrease in the amounts of FAA during the first 30 mins. Total quantity of amino compounds was found to have declined from 13,44 to 10,65 µmol (g dwt)$^{-1}$, but recovered to the initial level (13,43 µmol (g fwt)$^{-1}$ at 60 mins. The level of intracellular ammonia was much higher (0,766; 0,862 and 1,162 µmol (g fwt)$^{-1}$ at 0, 30, and 60 mins., respectively), whereas the level of FAA was similar at 60 mins. to that initially determined.

d) Levels of glutamine and glutamic acid showed only minor change during the first 30 mins. of the experiment (nitrate) but definitely decreased between 30 and 60 mins.
In the ammonium feeding experiment, the level of glutamic acid decreased to the original level, which also decreased slightly. Both glutamine and glutamate were found to increase again thereafter.

4.4. DISCUSSION

4.4.1. Nutrient Uptake

Nitrate and ammonium were taken up rapidly during the initial phase of experiment 4b, and uptake slowed down later on. This is due to a high flux of ions into the 'apparent free space' (AFS) saturating the intercellular spaces. Active uptake or facilitated uptake processes then largely supercede the initial AFS related uptake of nutrients. This non-linearity of uptake, detected by monitoring nutrient levels by the so-called perturbation method, has been measured for some phytoplankton (cited by Harrison & Druehl, 1981).

As was clearly established by the enriched-N uptake experiments performed on E. maxima, ammonium was the preferred inorganic nitrogen species at enriched N levels. It was taken up rapidly until near depletion levels were reached at the end of the experiment. This was shown by their respective rates of uptake : At a substrate concentration of 108,35 $\mu mol.dm^{-3}$ nitrate was taken up at a rate of 589 $\mu mol\ (kg\ dwt)^{-1}.hr^{-1}$. This contrasts markedly with the figures obtained for ammonium : At 81,6 $\mu mol.dm^{-3}$ the rate of uptake was 5020 $\mu mol\ (kg\ dwt)^{-1}.hr^{-1}$ - 8,5 times as fast as the uptake of nitrate at those concentrations. Considering the natural range of concentrations of inorganic nitrogen in seawater, these substrate concentrations were high and uptake can be assumed to be taking place at or near saturation levels (Probyn & McQuaid, 1985).

At ambient nutrient levels, however, uptake velocities for nitrate were consistently higher than for ammonium, reflecting the much lower ionic concentration of ammonium in seawater. Nitrate uptake velocities were approximately 1,45 to 1,55 times higher than those for ammonium. This makes nitrate the primary nitrogen resource of E. maxima, as established by Probyn & McQuaid (1985).

It remains to be established whether nitrate uptake was inhibited by the presence of ammonium, or whether the low uptake velocities observed for nitrate were similar even in the absence of other, possibly competing ions. It was found that when nitrate and ammonium were supplied separately, comparable trends occurred to when both forms were added simultaneously. In the medium with a high ammonium concentration, rapid uptake took place during the first 30 mins of the experiment and continued more gradually. Nitrate uptake did not seem to be affected by the presence of

high concentrations of ammonium : Despite its lower concentrations, it was taken up by the kelp linearly ; noticeably with only very little initial AFS uptake because of the reduced concentration level of nitrate. Conversely, a high nitrate concentration appeared to affect the uptake of ammonia since its levels remained constant after the initial increase.

The uptake of P was affected by a high concentration of nitrate, rather than by high ammonium levels with uptake proceeding much more consistently in the latter (compare Figs. 15 and 16). This may be explained by the fact that the nitrate ion has a negative charge like the various forms of phosphate (di- and trivalent anions) possibly causing a competitive interaction with the phosphate (which is present at much lower concentrations).
This hypothesis is reflected in the rate of uptake for P :
At a substrate concentration of 1.51 and in the presence of high nitrate the rate of uptake was 25 µmol P.(kg dwt)$^{-1}$.hr^{-1}, whereas when ammonium was substituted the rate was found to be 51 µmol P.(kg dwt)$^{-1}$.hr^{-1} (but also at a slightly higher substrate concentration of 1,63 umol.dm^{-3}). This could also indicate that the Vmax (the point of lowest substrate concentration at which saturation occurs)was very low. Nitrate and ammonium do not interfere with each other to any marked degree as was also shown by Bird (1976) and Topinka (1978), working with Gelidium nudifrons Gard. and Fucus spiralis, respectively. Uptake probably occurs at different sites involving different carrier systems. The preference of seaweeds for ammonium has been previously documented. DeBoer (1981) reported that uptake rates of ammonium generally exceed those of nitrate. However, Probyn & McQuaid (1985) demonstrated only a weak preference for ammonium in E. maxima, pointing out that kelp uptake rates for nitrate were almost an order of magnitude higher than for ammonium at

ambient nutrient levels. It is known from higher plants that nitrate uptake requires energy while ammonium uptake is an energy independent process (Bidwell, 1979). Even though the nitrogen requirements of the kelp could have been fulfilled adequately by the high input of ammonium, nitrate was still taken up.

Nitrate uptake at ambient levels by kelp was measured in discrete as well as continuous collection experiments (section 4.3.4.). During experiment 4a good consistency was achieved as far as the mode of uptake and disappearance ~~of~~ from the medium was concerned. The rate of uptake was determined as 299 µmol (kg dwt)$^{-1}$.hr^{-1}. This contrasts with the figure obtained 2 months later in late May when experiment 5 showed that between 95 and 113 µmol (kg dwt)$^{-1}$.hr^{-1} of nitrate were taken up. This represents a reduction by about ~~a~~ two-thirds in uptake capacity. When the experiment (6) was repeated in winter (June) the nitrate uptake rate had decreased even further to a level of 55 µmol (kg dwt)$^{-1}$.hr^{-1}, although the ambient concentration of nitrate was found to be very high (17,5 µmol.dm^{-3}) due to upwelling. Nitrate uptake thus possibly shows a seasonal trend. Uptake of nitrate was found to be lowest in August, when the rate was determined to be 38,8 µmol (kg dwt)$^{-1}$.hr^{-1}.

Looking at ammonium the same basic trend was noted, only less pronounced. However, the low concentrations often amplified fluctuations such that trends were not as clearly discernible and less reproducible than in the case of nitrate. The apparent increases in ammonium in some cases may have been due to remineralisation processes and deaminations caused by heterotrophs (Valiela, 1984). Compared to the nitrate uptake curves of the duplicate experiment, the ammonium monitoring showed almost opposite trends in the duplicates with no uptake

measurable in kelp B. It seems that baseline fluctuations were higher than anticipated registering simultaneous exudation/ heterotrophic deamination processes. If uptake did take place it was found to be irregular and for short time-spans only. From the data it appears that the uptake of ammonium was very low and often unmeasurable in winter, lending support to the concept of seasonality - influenced uptake rates. This was also found in the phosphate determinations :

Although only analysed for in May, June and August, the uptake rates for phosphate were between 9,14 and 16,2, then decreasing to 11.6 and finally decreasing again to only 4,31 µmol (kg dwt)$^{-1}$ hr^{-1}, respectively. Background concentrations did not correspond to uptake rates supporting the seasonality theory further. It appears, therefore, that aspects of the phenology and growth of Ecklonia maxima are closely linked to the uptake of inorganic nutrients as would be the case in higher terrestrial plants (Bidwell, 1979).

4.4.2. FAA Determinations

Once nitrate has entered the thallus, it is either stored (Chapman & Craigie, 1977) or directly utilized following reduction to ammonium that is combined with carbon skeletons to produce amino acids ; this taking place via the GS/GOGAT system (Miflin & Lea, 1977 ; Syrett, 1981). An increase in the levels of both glutamine and glutamic acid in tissues subjected to increased concentrations of externally supplied nitrogen would therefore be expected.

The data in Table 8 (exp. 4b) shows that glutamic acid concentrations were 1,38 times as high after 30 mins. than at the start of the experiment. Similarly, a 12% increase in the level

of glutamine in the fed vs. the untreated plant was recorded ; this corresponding to findings of Haxen & Lewis (1981) where pools of assimilatory amino acids (glutamine and glutamate) accumulated in Macrocystis angustifolia Bory.

The GS/GOGAT system is linked to oxo-ketoglutarate which enters the Krebs-cycle or undergoes transamination reactions resulting in the formation of other amino acids (Miflin & Lea, 1977). This explains the increased levels of almost all of the amino acids detected before and after the feeding experiment. Alanine was found to be prominent in the FAA analyses ; this having been discovered by Channing & Young (1953) in a variety of seaweeds. The concentration factor of alanine approached 1,6 in this study. Alanine has been proposed as a major nitrogen storage pool in brown algae (Wheeler & North, 1980). Also, the level of intracellular unbound ammonia was higher in the N-treated plant possibly indicating oversaturation.

An attempt has been made to calculate the amount of N assimilated during the first 30 mins. of the experiment. In the calculations it was shown (Appendix 4) that 55,51% of the TAA is taken up within 30 mins.; the figure being only 1,42% for the TAN. Based on a total amount of 15,17 mg nitrate and 11,42 mg ammonium per 10 dm^3 seawater, 3,669 umol (TAN+TAA) is assimilated per gram dryweight. By using the '%N' data from Table 8, it was calculated that 67,21% of (TAN+TAA) was assimilated into FAA within 30 mins.

The results obtained in experiment 7 did not show any marked accumulative trends of FAA during the 60 min. depletion-of-nutrient study. Accumulation of amino acid pools has been postulated to occur only under conditions of nutrient stress, ie. either nitrogen - starvation or generally slow nitrogen

metabolism (Thomas, 1983, cited by Lobban, et al, 1985). A lack of amino acid accumulation therefore implicates rapid changing of pools such as primary and secondary amino acids and polypeptides, necessitating the use of ^{15}N to quantify these pools (Lewis, pers. comm.). The temporary increase in FAA during the first thirty minutes of nitrate supplementation followed by a subsequent decrease could be explained by the assumption that nitrogen assimilation results in amino acid production followed by protein synthesis.

When ammonium was supplied, the amino acid pools are filled more rapidly (there are no intermediate nitrate reductase reactions) and are also depleted again much more rapidly. Ammonium nutrition in E. maxima is hypothesised to result in a higher flux of nitrogenous pools than during nitrate nutrition. The potential toxicity of ammonium may be responsible for inducing higher metabolic rates. High ammonium concentrations (>30 µM) can be toxic to some seaweeds (Haines & Wheeler, 1978). However, no evidence of ammonium toxicity could be detected in these short-term studies.

C H A P T E R

V

EXUDATION OF POLYPHENOLS AND UV - ABSORBANCE OF EXUDATES

5.1. INTRODUCTION

5.1.1. Polyphenols, Marine Humic Acids and Gelbstoff

A class of compound frequently encountered as DOM in seawater are the phenols and their polymeric derivatives, the polyphenols. The determination of the quantities and composition of these substances is of direct importance to elucidating the chemical nature of brown algal phenolic exudates in the inshore marine environment (Ragan & Jensen, 1977). Crato (1895, cited by Glombitza, 1981) determined these substances to be phloroglucinol or phloroglucinol derivatives. Brown algal polyphloroglucinols consist of 1,3,5 - trihydroxybenzenoid structural units that can combine in a number of ways to form large and refractory compounds. Glombitza (1981) has identified many of these highly hydroxylated phenols in both exudate and cellular contents of members of the Phaeophyceae. Depending on the type of bond structure, these substances can be referred to inter alia as phlorotannins or fucols.

Khailov (1963) reported that seawater contains an unidentified UV-absorbing substance derived from brown algae. Craigie & McLachlan (1964) determined that the yellow-coloured exudates of Fucus vesiculosus L. were of phenolic affinity ; their yellow colouration and UV-absorptive properties correlating well with those of Gelbstoff (see section 1.3.). Exudates of brown seaweeds and fluorescing DOM of inshore waters are very similar, with Gelbstoff possibly being formed by the interaction of algal polyphenols with proteinaceous material and carbohydrates (Sieburth & Jensen, 1969). Further change occurs by biological action (bacterial decomposition) and chemical processes, eg. hydrolysis and oxidation (Ogura, 1975). This leads to a reduction in molecular weight and complexity. Finally, these

highly refractory endproducts of various origins and compositions are collectively known as Gelbstoff.

5.1.2. Ultraviolet-Absorbance of Exudate Products

Because Gelbstoff absorbs light in the UV region of the spectrum it was thought to be ideally suited as an indicator of exudation and the accumulation of exudate products. Khailov & Burlakova (1969) measured exudation exclusively by this method, making use of a conversion formula relating absorbance to DOM concentration (see section 3.1.1). This method has subsequently been discredited as being too inaccurate and indiscriminate in DOM detection and quantification (Ragan & Craigie, 1980). These workers discovered that only a poor correlation exists between UV-absorbance and actual DOM concentrations, mainly because absorbance peaks of organic molecules are scattered throughout the UV part of the spectrum. Another reason for the failure of this technique is that the UV-extinction increases rapidly in seawater without additional DOM input due to oxidation reactions leading to discolorations. Consequently therefore, a UV-spectrophotometric analysis cannot be expected to yield accurate data about the actual concentrations of DOM in seawater ; estimates of DOM based on UV data are invariably too high in waters receiving exudate inputs. The method can, however, still be used where an approximate indication of DOM quantities is required and where sufficient time has elapsed between production/exudation of polyphenols and their final (non-changing) appearance as Gelbstoff (Carlson, 1982 ; Carlson & Mayer, 1983). Using a combination of colourimetric tests and UV-absorbance, definite evidence was presented that macroalgae-derived phenolic materials contribute to the DOM in sea surface slicks (Carlson, 1982). In a more recent study, Carlson & Carlson (1984) detected analogous trends

between UV-absorbance (at 280 nm) and phenolic contents of exudate, both being characterised by initial "pulses" of DOM release during the first 2-5 mins. High absorbance values are regarded with reluctance ; as a reason, reactions between exuded materials and other dissolved organic compounds were cited.

5.1.3. Measuring Polyphenol Release

Sieburth & Jensen (1969) made use of Brentamine Fast Red 2G for detecting and quantifying macroalgal polyphenols. In their study, they arrived at a figure for exudation of as much as 500-800 mg phenolic material per kg algal dryweight per day. Sieburth (1969) determined polyphenol exudation figures ranging from low in Chondrus crispus to high in Laminaria digitata (0,4 to 18,4 mg polyphenol per 100 g dw alga per day). These results were obtained in a flushing chamber simulating an open system, with values for other chemical parameters (carbohydrates and nitrogenous) being smaller and more inconsistent. Freshwater was observed to stimulate polyphenol release.

Working back from pooled estimates of green, red and brown algal exudation figures, Khailov & Burlakova (1969) determined 70% of total synthesis to end up as DOM. However, because UV methods were used exclusively, it is difficult to assess the percentage of polyphenols in total exudate. It presumably approached 30 % but could be as high as 51%, calculated using Sieburth & Jensen's polyphenol : DOM ratios.

Zavodnik & Jensen (1981) submerged intact specimens of Ascophyllum nodosum, Fucus vesiculosus and others in filtered seawater for 30 minutes. The concentration of phenolic compounds was colourimetrically estimated by the diazonium salt method (Sieburth & Jensen, 1969). The percentage of polyphenols to

total exudate was found to range from 10-30%, with A. nodosum producing less amounts of exudate than Pelvetia canaliculata (L.) Dcne et Thur. but relatively more phenols.
Carlson & Carlson (1984) found that there was a good correlation between DOC and phenolic measurements using a Folin-Ciocalteu assay : Approximately 17 - 19 % of the total amount of dissolved extracellular material derived from Ascophyllum and Fucus sp. consisted of polyphenols.

5.1.4. Aims

The influence of DOM derived from kelp on surrounding seawater during exudation was investigated by measuring UV-absorbance characteristics and polyphenol content of exudate.

5.2. MATERIALS AND METHODS

The Folin-Ciocalteu colourimetric assay was used in the determination of total polyphenols in seawater. Refer to section 2.7.3. for methodology.
The following pilot study was devised to monitor changes in UV absorbance in the surrounding seawater : A 100 g (fwt) piece of frond of E. maxima was immersed in 1,5 dm^3 of filtered seawater, taking care not to let the cut surface come into contact with the water. A spectral scan was carried out (see 2.4.4.) after 30, 60 and 90 mins. After that the kelp was removed. The exudate solution was kept at room-temperature for 18 hours. UV-spectroscopic analysis was done according 2.7.4.

5.3. RESULTS

5.3.1. Results of the Pilot Studies

5.3.1.1. Demonstration of UV-extinction Increase with Time

UV-absorbance was seen to increase with time in the pilot study (Fig. 26a-d) ; in particular, an absorbance peak at 280 nm becomes visible. UV-extinction still increased after the removal of the kelp.

5.3.1.2. Demonstration of the applicability of the Folin-Ciocalteu method

Fig. 27 represents the accumulation of polyphenols in both experimental buckets (exp. 1). After initial fluctuations (eg. at the 5 min. mark), therewas a definite increase in the quantities of polyphenols released by both kelps up to 60 mins. with levels increasing only slowly thereafter. At the end of the 90 min. experimental period, the small kelp-bucket system had accumulated a net 2,862 mmol PGE $(kg\ dwt)^{-1}$; the large kelp produced 1,634 mmol PGE $(kg\ dwt)^{-1}$.

5.3.2. Exudation as a Function of UV-Absorption

5.3.2.1. Continuous collection results

Fig. 28 demonstrates the UV-absorbance curve obtained during experiment 6. There was a higher absorbance at 5 mins and subsequent increase thereafter to 0,142 Absorbance Units (AU) at time 240 mins.

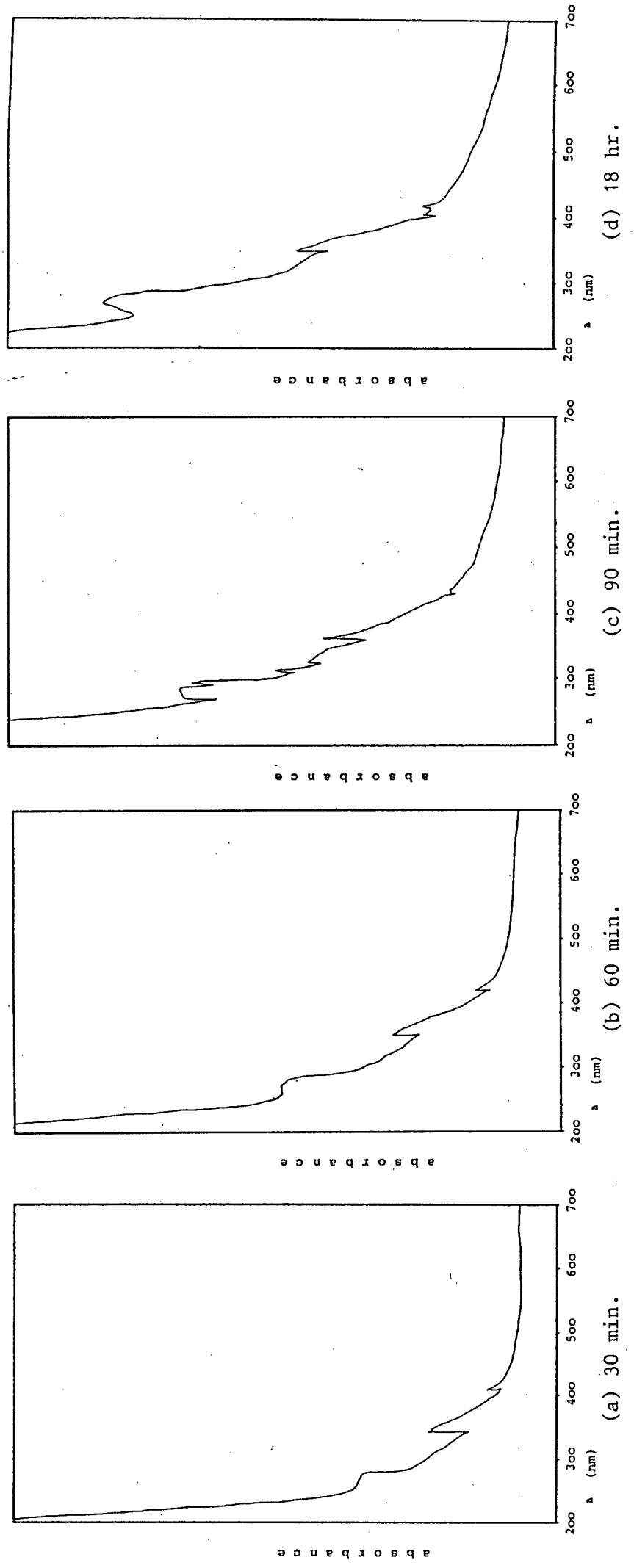


Fig. 26 a-d : Pilot study : Increase in UV-extinction of kelp exudates
After 90 mins. the kelp was removed.

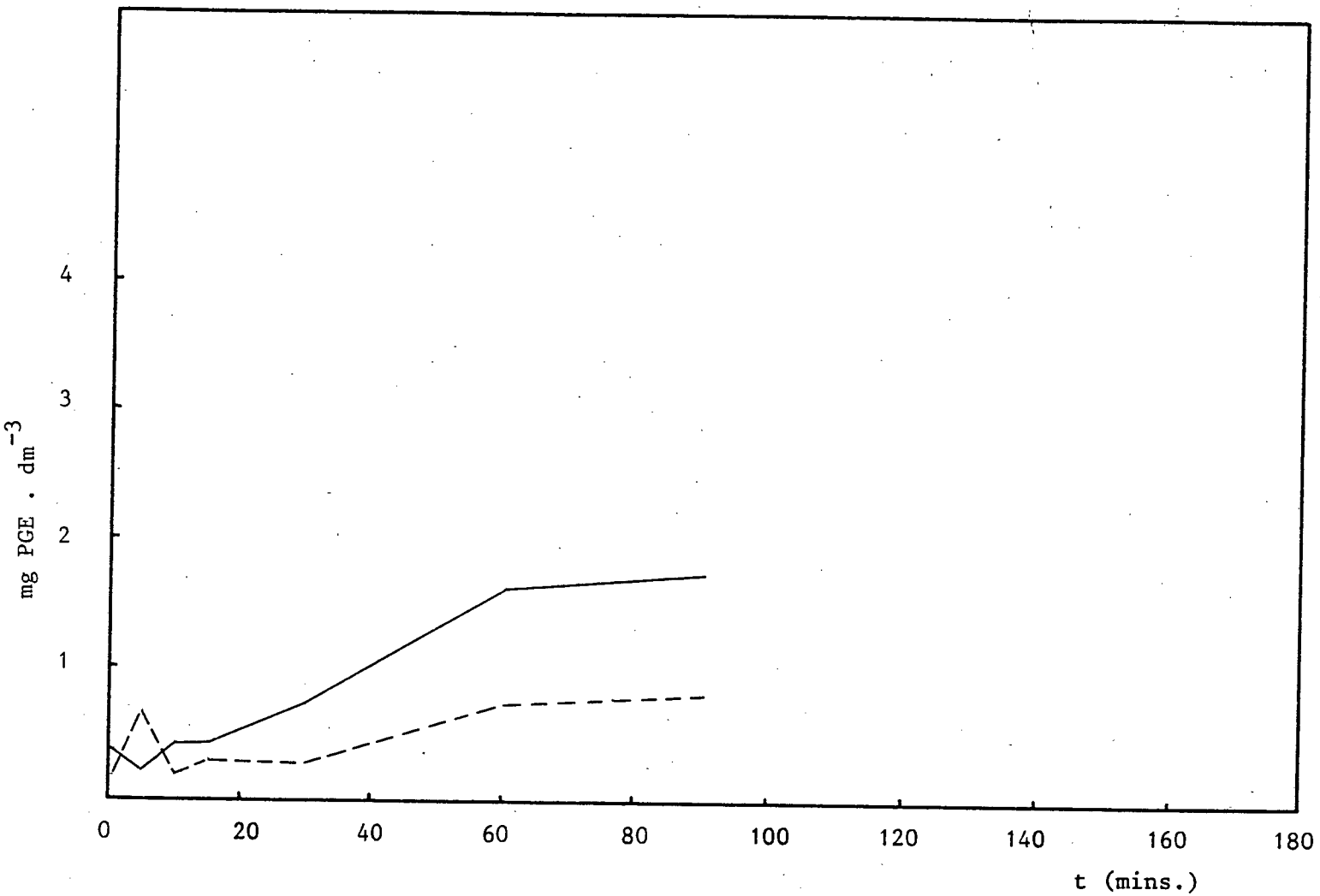


Fig. 27 : Exp.1 Polyphenol pilot study (F-C assay)

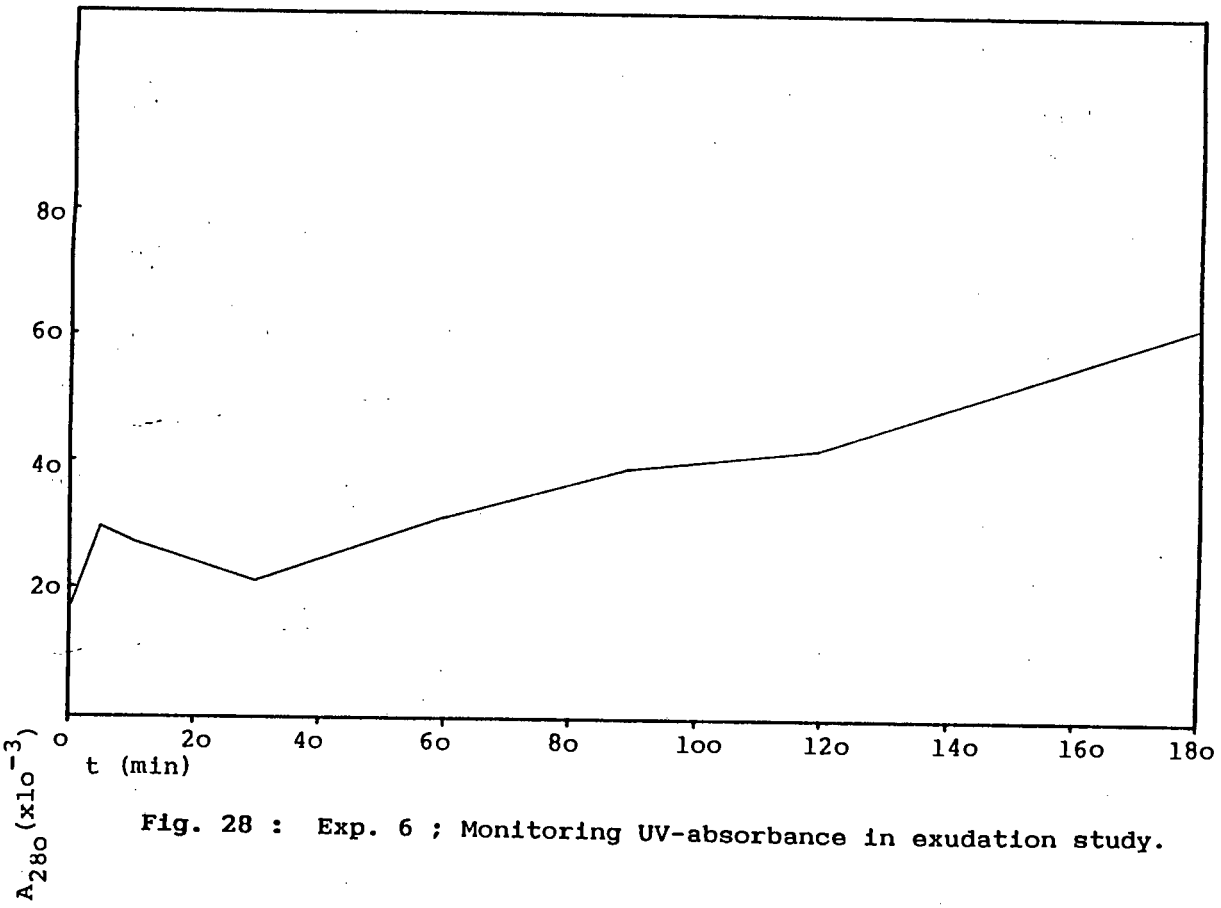


Fig. 28 : Exp. 6 ; Monitoring UV-absorbance in exudation study.

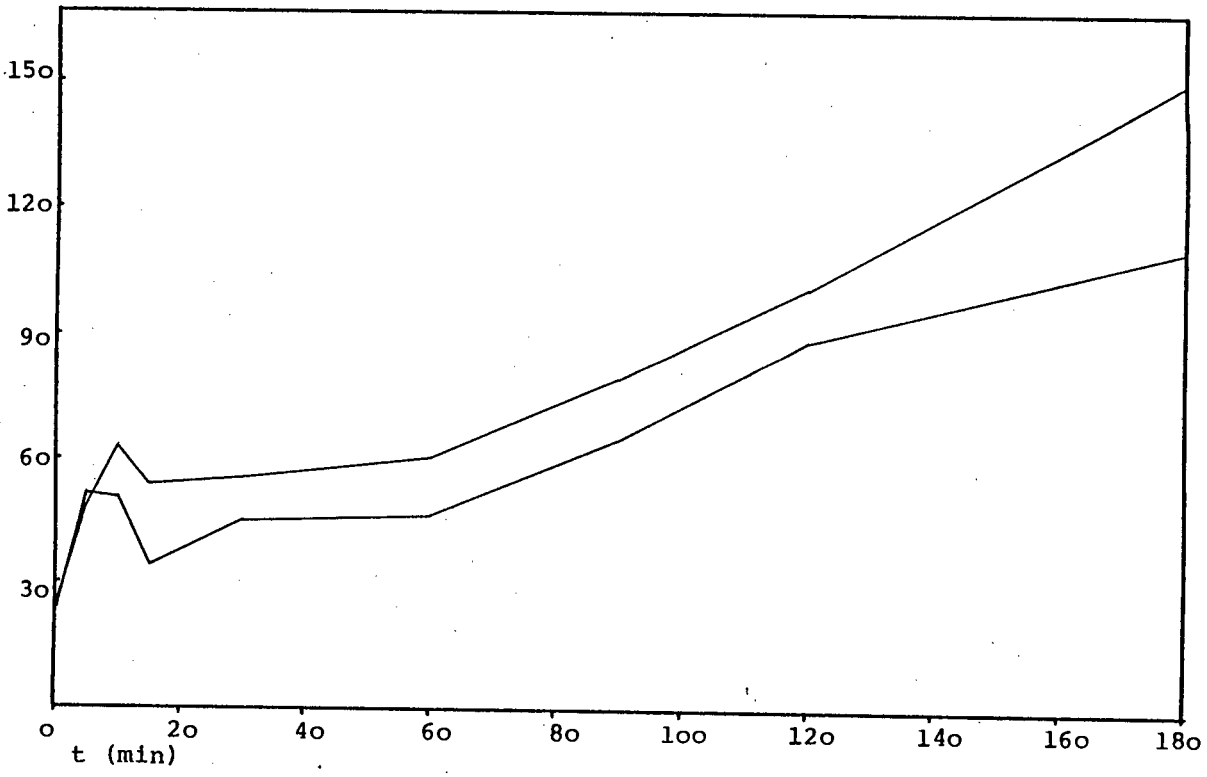


Fig. 29 : Exp. 7 ; Monitoring UV-absorbance in exudation study, kelp subjected to enriched nitrogen conditions (4.3.)

During experiment 7, two buckets were monitored, inter alia, for absorbance ; each containing an enrichment of either nitrate or ammonium (see section 4.3). As can be seen in Fig. 29, both figures are very similar and also with respect to 28 :
There were initial peaks at 5-10 mins.; followed by a gradual increase to a maximum absorbance value after 180 mins.

5.3.2.2. Discrete collection results

Most of the discrete measurements are characterised by a rapid initial increase in absorbance, often followed by an ~~in~~decrease or recovery to ~~higher~~ lower levels after 15 mins. (Fig. 30 - exp. 4a).
Less variation was noted in the UV-absorbance measurements in experiment 5 (in duplication). Initial DOM pulsing occurred at the first sample set (Fig. 31)

5.3.3. The Folin-Ciocalteu Assay for Polyphenols

5.3.3.1. Continuous collection results

Figs. 32 and 33 show the accumulation of polyphenols in exudates of E. maxima (exp. 6 & 7).
Fig. 32 was found to be similar to its corresponding Fig. 28 (section 5.3.2.1.). There was a large increase in the amount of polyphenols in the water between 180 and 240 mins., reaching levels of 260 µmol PGE $(kg\ dwt)^{-1}$. Likewise, Fig. 33 can be seen to correspond to Fig. 29. The experimental set enriched with nitrate had a lower polyphenol content initially, but ultimately contained more polyphenol than did the analogous ammonium set. Values at 180 mins. were 7,307 and 5,448 µmol $PGE.dm^{-3}$, respectively.

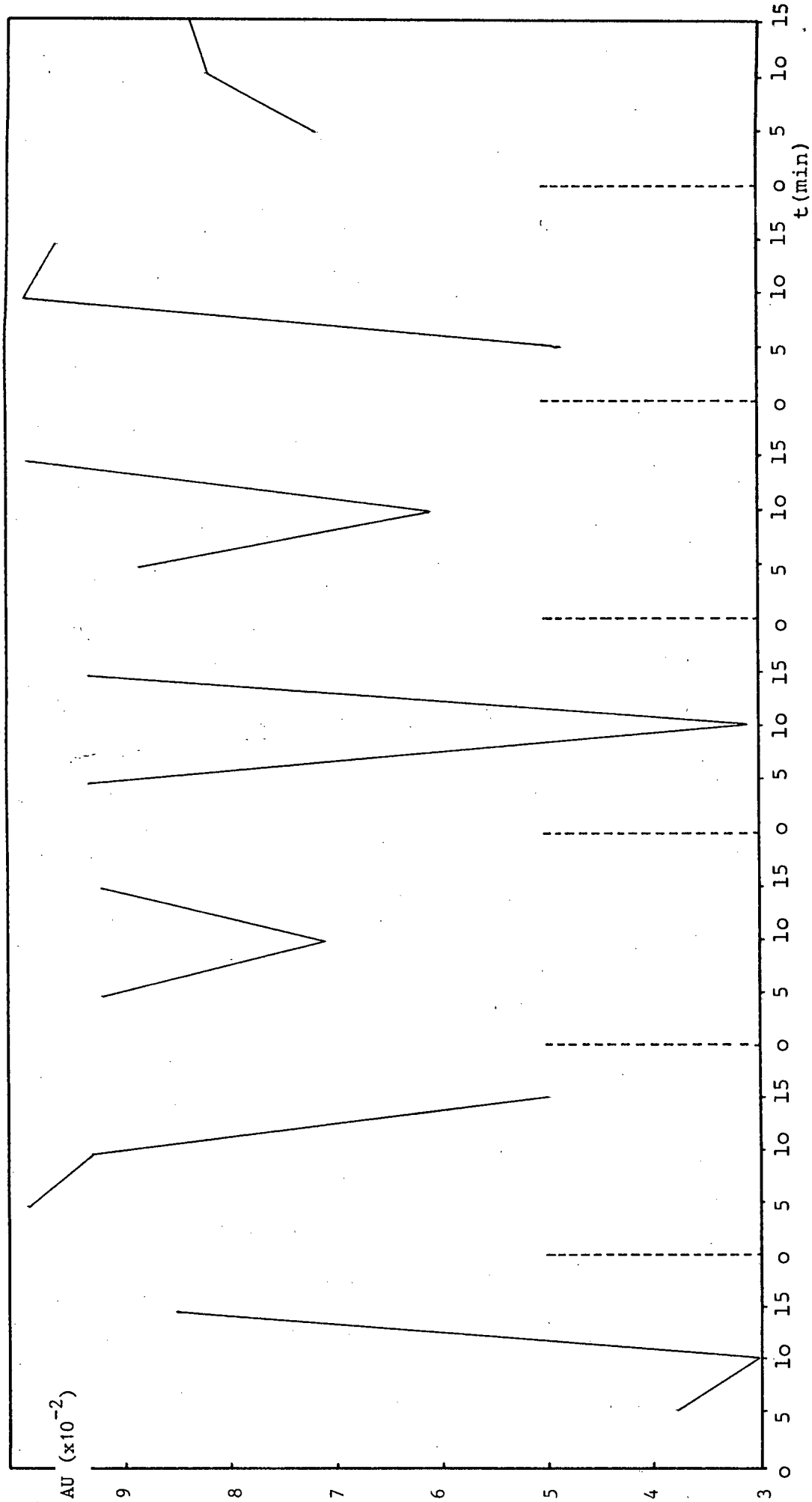


Fig. 30 : Exp. 4a ; Monitoring UV-absorbance (discrete coll.)

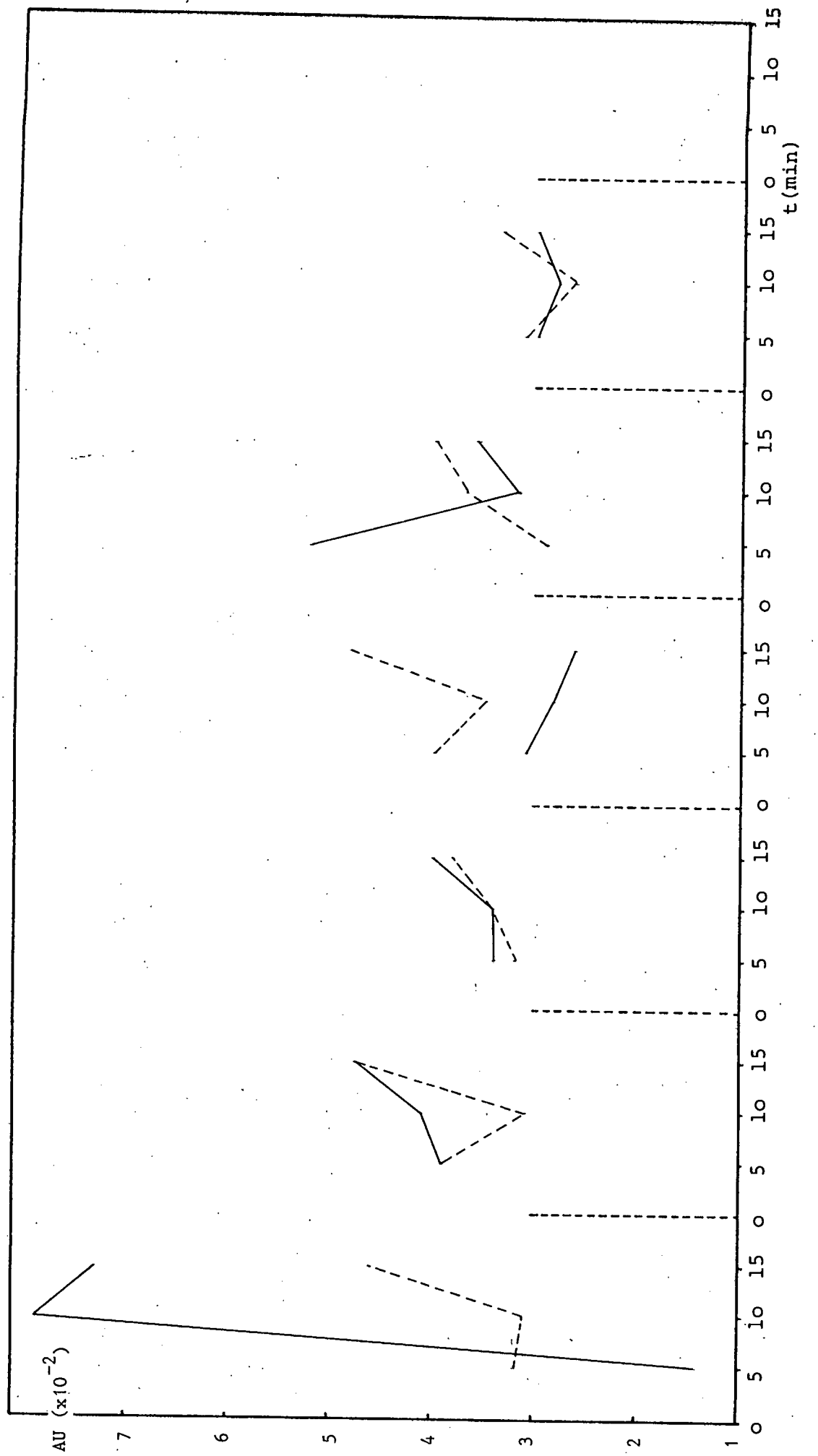


Fig. 31 : Exp. 5 ; Monitoring UV-absorbance (discrete coll.)

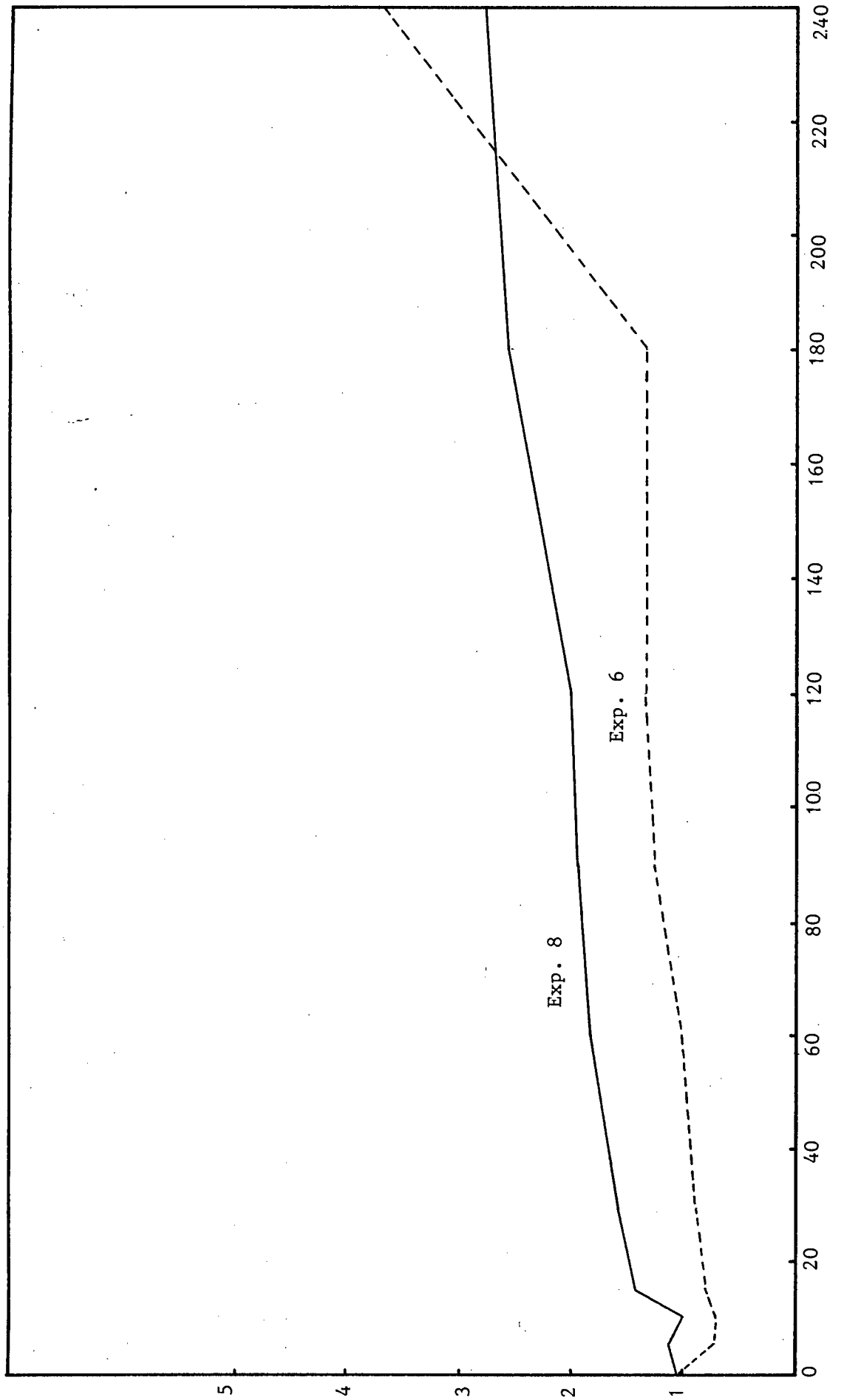


Fig. 32 : Exp. 6 & 8 ; Monitoring of polyphenols (F-C assay)

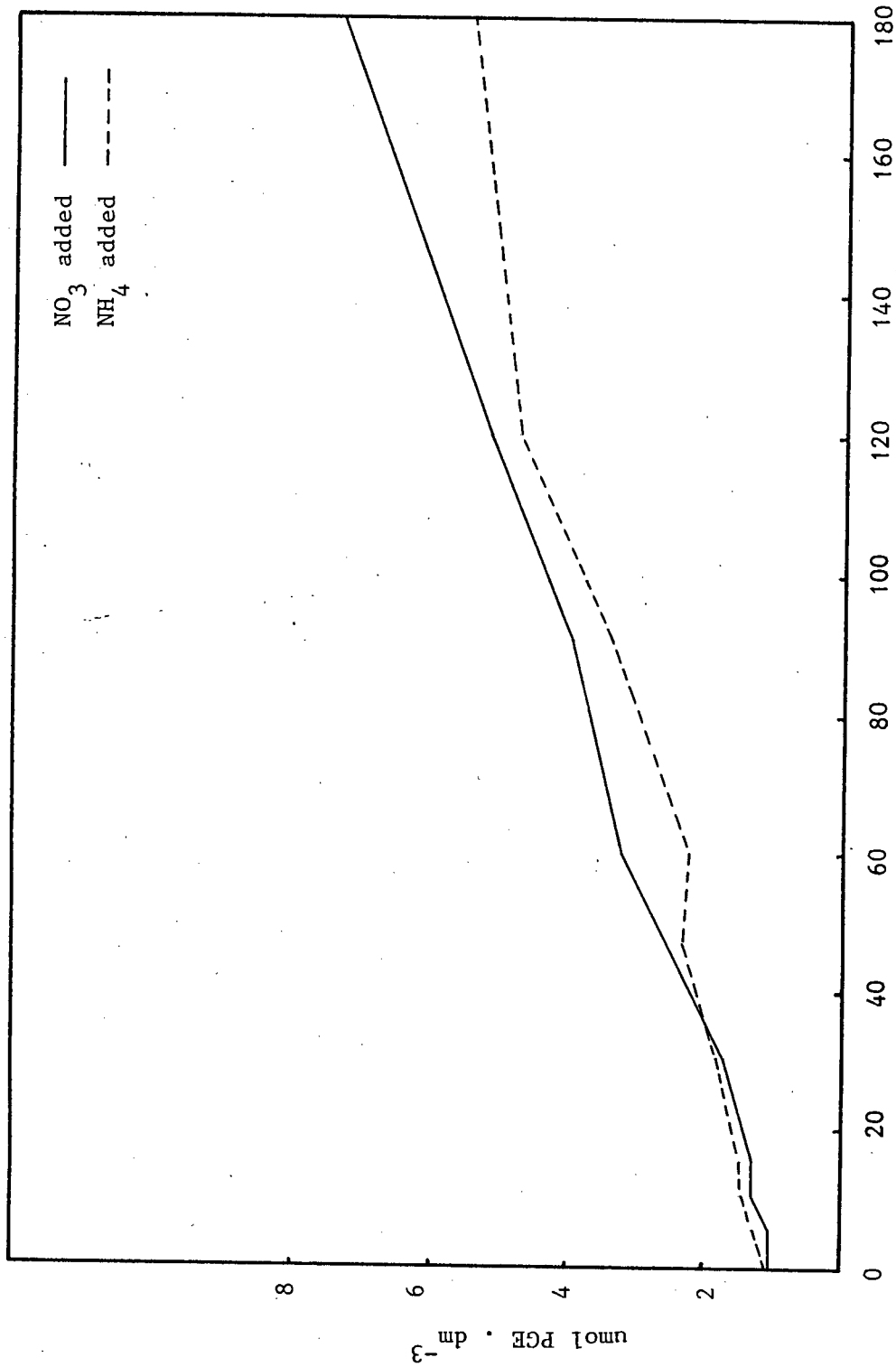


Fig. 33 : Exp. 7 ; Monitoring of polyphenols for N-enrichment experiments

The following rates of polyphenol release were calculated from the data :

Experiment 6 (6-6-86) = production of 51,36 µmol PGE (kg dwt)$^{-1}$ hr^{-1}.

Exp. 7 (8-8-86, nitrate) = production of 99,07 µmol PGE (kg dwt)$^{-1}$ hr^{-1}.

Exp. 7 (8-8-86, ammonium) = production of 68,88 µmol PGE (kg dwt)$^{-1}$.hr^{-1}.

Less polyphenols were released during experiment 8 (20-8-86). Only 19,72 µmol PGE (kg dwt)$^{-1}$.hr^{-1} were liberated by the kelp.

5.3.3.2. Discrete collection results

Fig. 34 (exp. 4a) showed irregular peaking at 5 and 10 mins. during the first sample set. Thereafter the production of extracellular phenolics was more stable with a release of between 8 and 9 µmol PGE.dm^{-3} within 5-10 mins. for the rest of the experiment. There was a rapid decrease in polyphenol content of the seawater after the initial peak.

Data obtained in experiment 5 show that there was a close correlation and reproducibility in the two sets : In both sets the production of phenolic material decreased with time. There was a steady decrease from about 2,5 to about seawater backgroud level of approximately 0,7 µmol PGE.dm^{-3} in "A". In "B", the background level was measured to be higher at 1,5 µmol PGE.dm^{-3} (Figs. 35a & b). These values, calculated on a kelp mass basis, correspond to the liberation of the following quantities of polyphenols :

A was found to release 100,73 µmol PGE (kg dwt)$^{-1}$.hr^{-1}.

B liberated 117,43 µmol PGE (kg dwt)$^{-1}$.hr^{-1}.

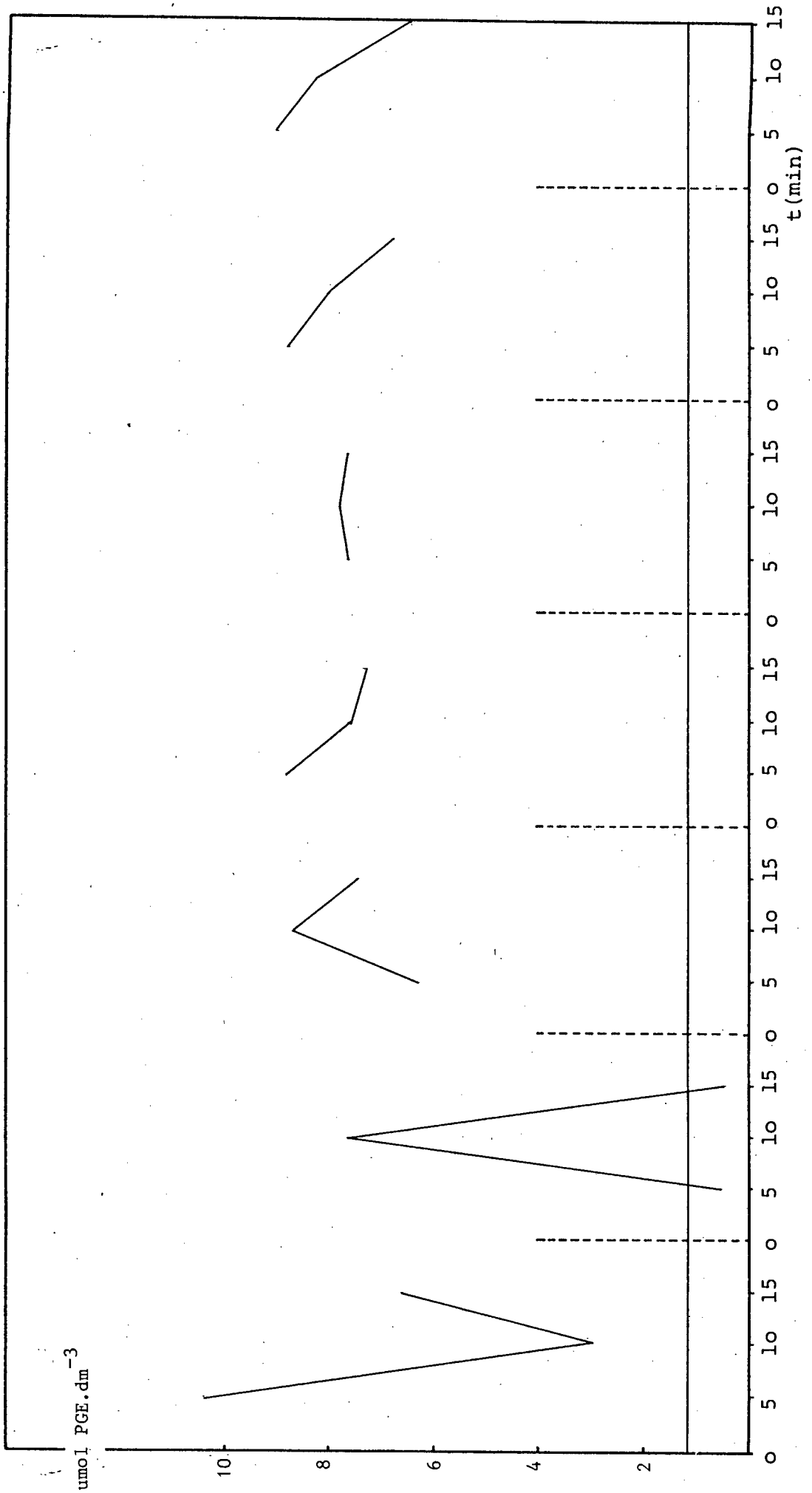


Fig. 34 : Exp. 4a ; Monitoring of polyphenol concentrations (discrete coll.)

Background concentration indicated by line.

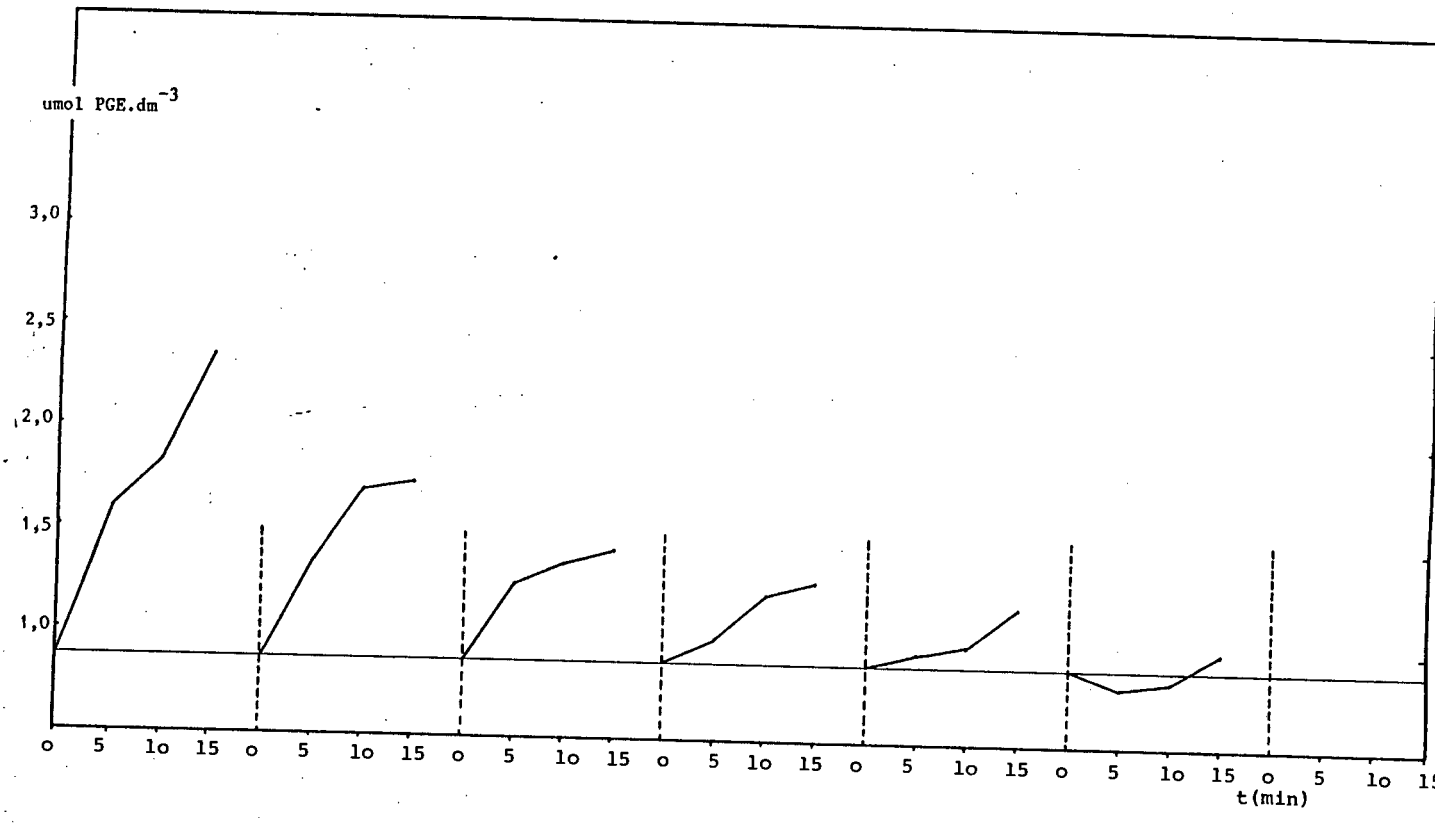


Fig. 35a : Exp. 5 ; Monitoring of polyphenol concentrations (discrete coll. kelp A)

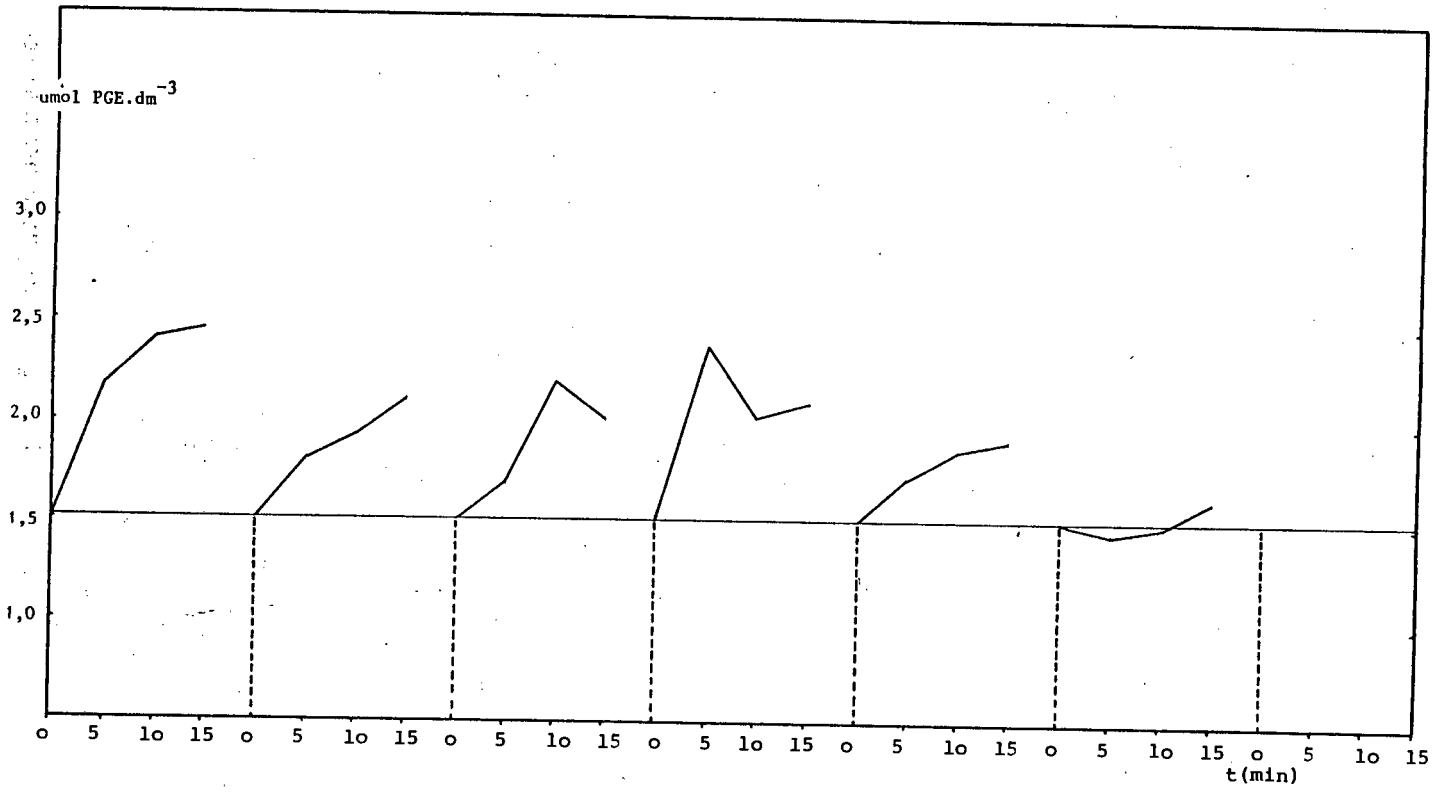


Fig. 35b : Exp. 5 ; Monitoring of polyphenol concentrations (discrete coll. kelp B)

Background concentration indicated by line.

5.4. DISCUSSION

The preliminary results obtained in the pilot studies have focussed on three aspects of this type of research. Firstly, DOM of algal origin does accumulate in the surrounding seawater and can be traced by techniques such as polyphenol analysis and UV-absorbance. This is because the refractory nature of these compounds allows them to persist and accumulate in the water column to a greater degree than the DON (Brylinsky, 1977). Since the DOM consisting of polyphenols and their derivatives do not contain any nitrogen (Glombitza, 1981), they are less likely to be immediately incorporated into marine bacteria and other consumers. Secondly, the lack of a suitable standard makes the UV-absorbance technique less useful in monitoring exudation patterns and can only be used as an analytical tool on a relative basis. Thirdly, it was shown (section 5.3.1.1.) that UV-absorbance increased after the sorce of DOM input was removed, corresponding to the findings of Ragan & Craigie (1980).

An important aspect of polyphenol and UV-absorbance monitoring was the similarity in terms of the patterns characteristic for both analyses. There was the rather large initial peak at around 5-10 mins., probably signalling a sudden release of UV-detectable DOM in continuous as well as discrete collection methods. Often this phenomenon is reflected by a similar trend in the polyphenols. This seems to give a definite indication that most of the UV-absorbing molecules are in fact phenolic in origin, corresponding to the findings of Sieburth & Jensen (1969). The pH of the medium influences the formation of Gelbstoff (Sieburth & Jensen, 1969). Since exudation is often accompanied by a decrease in pH resulting from the liberation of organic acids (Fogg & Boalch, 1958 ; Brauer, 1984) the quantitative detection

of polyphenols could be influenced.

That exudation was highest and most active during the first few minutes of the experiments is consistent with the results by Carlson & Carlson (1984) who stressed the fact that the large initial pulsing is evident in all their discrete collection measurements. This can obviously lead to uncertainties when exudation rates are to be assessed (Chapter 6). Conducting the discrete collection experiments has demonstrated that the initial pulsing of DOM (and DON - see section 3.3) occurs as a response to a "new" environment. It could be speculated that a combination of aeration, experimental stress on the plant and fresh ambient seawater could illicit the observed response.

During continuous collection measurements, however, a definite accumulation of poyphenols in the surrounding seawater was seen to have taken place. This can be taken as an indication that exudation of less biodegradable substances leads to linear accumulation and can be assessed in that manner.

There was a distinct seasonality in terms of quantities of polyphenols released. Much more phenolic material was liberated during the experiment in March than in May, analogous to the observations of both DON release and nitrogen and phosphorus uptake. This trend is also shown by the continuous collections : The least amount of material was found to be liberated in August (parallel to the least amount of uptake), but the most in March.

C H A P T E R

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DISCUSSION AND CONCLUSIONS

This study has highlighted several important findings ; arguably the most important being that exudation only rarely occurred as a steady process of DOM release as was often assumed in the past. Irregular fluctuations and pulsing patterns of DOM on a temporal scale of minutes were observed to take place during the experiments, and were recorded in DON, DOC, DFAA, polyphenol and UV-absorbance measurements. These pulses can arise as a net result of a variety of combining factors :

i) Photosynthetic productivity plays a role by being directly responsible for determining seasonality and growth patterns. Generally, higher rates of production (as in spring and early summer) are synchronized with higher rates of DOM liberation ; a point discussed in more detail later on.

ii) There is the possibility of detecting and interpreting experimental artifacts caused by shock-response. It should be taken into account, however, that Ecklonia maxima is frequently subjected to emersion during swells and therefore short-term exposure in its natural habitat. Consequently, it appears that the chance of recording artifacts with the devised experimental design is small, especially when considering that the time span between collection of kelp and experimentation had been kept to a minimum. An initial pulse of DOM was a consistent feature throughout most of the experiments. This could be due to the new environment the kelp was subjected to. The fact that initial pulses were described previously during exudation experiments on attached in situ fucoids (Carlson & Carlson, 1984) would imply that using detached plants does not prompt any additional stress on the seaweed. It was also noted that the kelp appeared to be photosynthesising and physiologically functional. This could seen by the formation of oxygen bubbles on the fronds. With some justification, analogous conclusions between the experiment and

the system as a whole in its natural state can be drawn. This should be mentioned in defence of the experimental design and the possibility of obtaining non-reproducible artifacts.

iii) Exudation can be mediated bacterially or involving the epidermis of the kelp. This was demonstrated indirectly by the the addition of antibiotic substances : Antibiotic agents did not succeed in controlling the bacteria in the water column without adversely affecting the physiology of algal epidermal cells. From the observations on the action of these substances (chloramphenicol is also a respiratory-chain uncoupler), it can be concluded that exudation may be an active, energy-dependent process, and not only due to simple leaching. This would also explain rapid liberation of DOM during pulsing intervals.

iv) Low heterotrophic activity in the system combined with a low bacterial count resulted in increased rates of DOM accumulation. It was discovered that bacterial activity was closely related to bacterial numbers. The appearance of pulses of DOM in the exudate coincided with a low heterotrophic activity, taking a certain lag-phase into account. Polyphenols, as part of the DOM pool, tended to accumulate in exudate to a greater extent than other forms of DOM for mainly two reasons : Polyphenols are refractory compounds not quickly degraded. Also, they do not contain N which makes them less desirable to marine heterotrophs.

Pulses of DOM were found to occur at irregular intervals in different plants. This made a statistical treatment of exudation difficult and less meaningful, as was shown in the triplicate experiment. Also, there is the inherent complexity of a kelp-seawater system that contains heterotrophic and planktonic elements. As was shown by subsequent filtered seawater experiments, the variability of the system can be successfully decreased but logistic problems of setting up more than two

concurrent exudation (or depletion-of-medium) experiments are a hindrance to replication in continuous and especially discrete collection modes. Therefore, the question arises whether pulsing occurs *in situ*. To test this it is recommended to carry out experiments involving plastic-bag enclosures similar to those used by Probyn & McQuaid (1985).

The depletion-of-nutrient uptake experiments were successful in combining exudation and uptake monitoring using the same experimental set-up. Studying the uptake of inorganic nitrogen and phosphate it could be demonstrated that ammonium was the preferred form of nitrogen under enriched nutrient conditions, if uptake velocities are taken as an absolute measure of preference. However, nitrate is the main form of available nitrogen in the inshore marine environment. This fact is reflected in the observation that nitrate uptake velocities were higher than those for ammonium at ambient nitrogen levels. This is understandable when considering the respective concentrations of nitrogen in seawater : Natural levels of nutrients in seawater are subject to fluctuations depending on the time of year and upwelling. Off the coast of the south-western and southern Cape Province, nutrient-rich waters are brought inshore by a combination of the actions of the Benguela current and offshore winds causing a replacement of coastal, warmer and more nutrient - depleted seawater. Depending on upwelling, the concentrations of nitrates and phosphates can vary considerably : oceanic waters commonly contain 0.8 to 1.5 $\mu mol.dm^{-3}$ of P with higher values (up to 6) along the coast. Nitrate can occur throughout a wide range of concentrations from about 4 in depleted waters to 18 $\mu mol\ N.dm^{-3}$ in newly upwelled seawater (Chapman & Shannon, 1985).
Ammonia present at low levels (0-3 $\mu mol\ N.dm^{-3}$) does not fluctuate in synchrony with upwelling to the same extend as do

nitrates and phosphates. This is due to the fact that ammonium in the marine environment is almost exclusively regenerated nitrogen, while nitrate input due to upwelling presents "new" N to the system (Eppley & Peterson, 1979).

Higher uptake velocities of ammonium are probably due to its independence of energy-requiring uptake mechanisms and a higher affinity for AFS related uptake (Bidwell, 1979). The results of this study confirmed the findings of the Probyn & McQuaid (1985) study on E. maxima uptake ; namely that uptake does not show signs of saturation beyond 20-30 µmol $N.dm^{-3}$, which is anomalously high for a natural situation. However, even at high enriched concentrations of ammonium, nitrate uptake continued to take place. This can be due to a variety of reasons : Firstly, as has been demonstrated in higher plants, a combination of these two forms of nitrogen are desirable to seaweeds and are likely to act in synergy. Secondly, the frequently very low levels of ammonium do not constitute a reliable nitrogen source available for growth. Thirdly, there is the documented ability of seaweeds to store nitrate reserves for times of nitrogen depletion in the water (Chapman & Craigie, 1977). Nitrate does not have to be assimilated into amino acids immediately because it is not toxic in the plant body like ammonium. The level of nitrate in the waters of the coasts of the south-western Cape Province, although subject to wide-ranging fluctuations, never reaches near-total depletion levels as is the case in coastal waters along the east coast of Canada (St. Margaret's Bay). The ecophysiological implications of this could be described as follows : The reduced need for nitrate storage could be responsible for a reduced nitrate uptake capacity at certain times of the year. To fulfil nitrogen requirements, the kelp takes up ammonium preferentially. Although Probyn & McQuaid (1985) have shown that E. maxima stores

nitrate, there could be a difference in nitrate uptake capacity and metabolism between South African seaweeds strongly affected by upwelling and mostly regular N supply and northern hemisphere species possibly subject to more stringent temporary nitrogen limitations.

Nitrate and ammonium uptake do not inhibit each other competitively. This can be explained by the difference in ionic charge, causing different carrier-molecule uptake sites to be activated. This hypothesis is supported be the finding that phosphate uptake was influenced by the presence of enriched nitrate but not ammonium : Phosphate and nitrate are both anionic species competing for the same uptake site in E. maxima.

The DFAA analysis has shown that the amino acid composition in inshore seawater is influenced by the presence and amino acid composition of the main producers. This was shown by the prominence of alanine in both kelp tissue and in surrounding seawater. Further, glutamic acid appeared in the exudate, but was not initially present at detection limits. Together with the overall increase in alanine, which has been postulated as a nitrogen-storage pool in seaweeds (Wheeler & North, 1980), the presence of these amino acids would indicate the operation of the GS/GOGAT system of nitrogen assimilation leading to an increase in total N content (Syrett, 1981). Generally, the amino acid profile of inshore seawater is determined by two factors : Extracellular DOM is derived from producers and bacterial breakdown products of heterotrophic utilization, where the profile can be affected by the prevailing proteolytic strain of bacteria. Because of the intermediate action of heterotrophs and rapid turnover processes, amino acid profiles appear less useful for assessing the DON contribution of main producers with known

amino acid compositions.

Finally, the question can be asked whether the exudation patterns determined in this study can be used for calculating exudation rates and their contribution to the annual energy budget of Ecklonia maxima assessed. The presence of pulses makes it difficult to decide whether to include these in the estimations since the question of laboratory artifaction has not been completely resolved. As a second point, it should be reiterated that DOM exudation profiles represent the net result of production and utilization. The possibility of bacterial lysis and leaching processes has not been taken into account, but is assumed to be negligible. The presence of phytoplankton in experiment 3 would make the calculation of annual exudation rates meaningless.

It was assumed that the average E. maxima kelp turns over its annual biomass about four times a year (ie. one kg of kelp produces four within a year - Jarman, pers. comm.). These figures are based on the hole-punching method measuring growth by frond elongation and can vary considerably between three and eight, depending on a variety of environmental factors.

Looking at the production of DON during continuous collection experiments carried out in early June (exp. 6) and late August (exp. 8), figures of 0,55 to 1,11 % and 0,1 to 0,2 % of total annual production released as DON (measured as total N) were calculated, respectively. On the other hand, figures obtained during discrete collection experiments were much higher, primarily due to seasonality and the presence of more pulses : 20,5 to 40,9 and 11,5 to 22,9 % of total annual production were calculated for March (exp. 4a) and May (exp. 5 - pooled), respectively. Discrete and continuous collection modes are responsible for yielding differential rates of release and are

not comparable on the same level.

Initially, more DON and other DOC appears to make up the total DOM pool than polyphenols. After release, however, the ratio changes continually in favour of the polyphenol fraction due to the preferential heterotrophic utilization of DON. The results of the May experiment (5) lead to the calculation of the following figures : Between 1,78 and 3,56 % (kelp A) and 4,11 to 8,22 % (kelp B) of the total annual production are liberated as polyphenols. As in the case of DON production, the figures were found to be lower for continuous collection experiments : In exp. 6, 0,92 to 1,84 % and 1,4 to 2,8 % (exp. 8) were released annually. The polyphenol measurements for experiment 4a were found to be very high and probably represent experimental error (51 to 102 %). The N enrichment experiment figures of early August (7) are within the range reported above (1,22 to 3,5 %).

These annual rates of extracellular release are only tentative. Not only do factors such as recording of pulses, seasonality or experimental type play a role ; the uncertainties involved in estimating the annual production of E. maxima affect exudation rates to a large extent. Although a figure of 1:4 of kelp production was chosen, it could be as high as 1:7 (Jarman, pers.comm.), altering the calculated rates by half to a third, reducing the rather high rates of some experiments but making others, especially the winter measurements, approach zero. The fact that these figures are all within the limits of other rates reported in the literature (section 3.1.1.) would indicate the validity of the experimental designs and the laboratory methodology that were adopted in this study.

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A P P E N D I X

Appendix 1a

Laboratory method for the determination of total N by alkaline peroxodisulphate oxidation (adapted from Nydahl,1978 and Mostert, 1983).

Solutions :

Made up according to Nydahl (1978).

Method :

Add 5 ml of alkaline peroxo solution to 5 ml seawater sample in a 100 x 15 mm low speed centrifuge tube. The mixture is heated in a digestion block for 90 mins. Upon cooling, a white magnesium hydroxide precipitate settles which is further compacted by low speed (1000 rev/min.) centrifugation for 3 mins. A sample is taken using an automatic pipette to fill the AutoAnalyzer cups. If necessary, dilute samples with dist. water.

Remarks :

Contrary to the method of Nydahl and Mostert, where acidic Tris-buffer or HCl are used, respectively, to rid the solution of precipitate, it was found that centrifugation is a preferable method because no additional N-load is placed on the sample.

Appendix 1b

Laboratory method for the determination of polyphenols in seawater (Modification of method by Box, 1983).

Solutions :

1) 1 M Na - citrate
2) Folin - Ciocalteu (Merck No. 9001)
3) sodium carbonate [sat.] (200 g/l)

Method :

Pippette 10 ml sample into 100 x 15 mm test tube. Add 2 ml Na-citrate solution, 0,6 ml sodium carbonate and 0,5 ml undiluted Folin-Ciocalteu reagent. Mix by inverting. Let stand for 4 hours for colour development. Standards are made up in artificial seawater (Strickland & Parsons, 1972) using phloroglucinol (BDH) in the following concentrations : 2, 5, 10, 20 uM. Read at 750 nm in 40 mm cells.

Remarks :

The sodium citrate has the effect of preventing the precipitation of magnesium hydroxide which accompanies the increase in pH upon the addition of alkaline sodium carbonate. The precipitate was found to interfere with the method as described by Box (1983).

Artificial Seawater prepared according to Strickland & Parsons (1972).

Appendix 1c

CHEMICAL OXYGEN DEMAND (COD) IN SEAWATER

from : Michel,P. (1972) Mesure de la demande chimique en oxygène dans l'eau de mer. Rev. Trav. Inst. Peches marit. 36 : 361-365

1. Reagents :

1.1. Titrant :
Dissolve 9.8g $FeNH_4(SO_4)_2.12H_2O$ in distilled water, add 2o ml concentrated H_2SO_4 and make up to 1 litre.

1.2. Oxidant :
Add 125 ml conc. H_2SO_4, loo g $HgSO_4$, and 1 g of Ag_2SO_4 to 75o ml H_2O. Cool and add 1.226 g $K_2Cr_2O_7$. Make up to 1 litre.

2. Method :

Use a 25o ml conical flask.
Add : lo ml sample or artificial seawater blank,
lo ml oxidant,
25 ml conc. H_2SO_4 under constant agitation.
Cover with watchglass and allow to stand for 2o mins.
Add loo ml H_2O

Titrate using 1 drop of Fe-ortho-phenanthroline as indicator, available commercially as Ferroin solution.

$$COD = 2o \text{ (blank tiration - sample titration) mg } l^{-1}O_2$$

3. Remarks :

3.1. The precipitate of AgCl formed on addition of the oxidant should easily dissolve upon addition of the H_2SO_4.

3.2. The same bottle of H_2SO_4must be used throughout due to the presence of oxidizable material in even very pure sulphuric acid.

3.3. The blank consists of 33g/l NaCl + distilled H_2O. Standards are made up in this using the following stock solution :
looo ppm COD = o.4686 g glucose to 5oo ml artificial seawater.

Appendix 1d

Preparation of tissue samples for the analysis of non-protein amino acids (Ethanol extraction).

Method :

Upon analysis, the tissue frozen in liquid N_2 is crushed into gravel-size pieces which are subsequently subjected to a mild desiccation treatment at 50 C in a draught-oven. The resulting dry flakes are then milled in a GE electric grinder (meshsize £20). Accurately weigh out between 1,5 and 3,0 g of ground tissue and add 50 ml of 80% ethanol in an Ehrlenmeyer flask. Take sample to dryness by blowing it down. Resuspend and dilute to 40 ml, adding 10 ml of a 2% Brij-35 solution. Petroleum ether (5 ml) is poured onto the extract which is frozen and the ether poured off. The sample is stored at 0 C for 48 hours before ultra-filtration (Whatman GF/F and Millipore filtration turret) and analysis.

Table : Bacterial Numbers and Biomass Table B in $pg.dm^{-3}$

	Bacterial Numbers ($10^{}$)	Biomass
0 mins		
A1	11.73	7.37
A2	25.90	12.19
A3	36.16	20.42
B1		
B2		
B3		
C	37.48	18.56
5 mins		
A1	17.39	12.31
A2	37.28	29.90
A3	57.08	34.75
B1		
B2		
B3		
C	39.44	17.70
10 mins		
A1		
A2	17.25	10.18
A3	33.38	25.75
B1	16.27	8.94
B1		
B3		
C	80.39	533.52
15 mins		
A1	54.24	25.94
A2	67.54	25.67
A3	169.82	136.01
B1	123.39	47.22
B2		
B3		
C	31.91	16.29

30 mins

A1	44.81	29.62
A2	25.74	18.08
A3	64.02	41.26
B1	10.89	6.28
B2		
B3		
C	11.14	6.29

60 mins

A1	11.87	5.41
A2		16.02
A3	29.95	22.49
B1	23.60	13.25
B2		
B3		
C	32.74	20.58

90 mins

A1	26.53	15.97
A2	79.17	29.81
A3	45.25	31.42
B1	30.15	16.35
B2		
B3		
C	39.43	17.44

120 mins

A1	29.66	21.60
A2	61.43	34.90
A3	40.41	28.11
B1		
B2		
B3		
C	26.87	16.88

150 mins

A1	25.90	14.05
A2	9,62	4.95
A3	73.69	43.22
B1	36.65	20.91
B2		
B3		
C	25.07	12.02

180 mins

A1	22.14	13.67
A2		
A3	53.12	30.49
B1	26.73	12.04
B2		
B3		
C	8.45	4.47

210 mins

A1	24.28	16.02
A2	37.29	16.78
A3		
B1	33.08	19.12
B2		
B3		
C	34.84	20.86

240 mins

A1	36.51	18.99
A2	9.09	6.28
A3	77.95	36.56
B1		
B2		
B3		
C	39.44	21.17

Exp. 3

0 mins

	[Ammonium]	x SE	[Nitrate]	x SE	[Total N]	x SE	[DON]	x SE %
A1	2.06	1.47	10.28	5.51	13.64	14.16	0.76	6.9
A2	1.07	0.30	2.41	2.42	16.94	1.55	13.64	3.69
A3	1.27		3.85		11.60		6.48	48.7
B1	5.08	5.16	4.39	3.80	2.66	2.89		
B2	4.79	0.24	3.31	0.32	3.05	0.12		
B3	5.62		3.71		2.95			
C	0.75		3.88		10.64		6.01	

5 mins

A1	0.67	1.19	5.00	4.51	11.86	12.09	6.19	6.38
A2	1.52	0.26	4.31	0.25	12.66	0.29	6.83	0.22
A3	1.39		4.22		11.74		6.13	52.7
B1	2.57	3.11	6.08	5.51	3.94	4.74		
B2	3.--	0.34	5.41	0.31	6.68	0.98		
B3	3.75		5.03		3.61			
C	1.53		5.11		1.79		0.26	

10 mins

A1	1.32	1.04	6.77	4.54	53.75	43.90	45.66	38.31
A2	0.77	0.16	3.50	1.11	48.43	7.36	44.16	6.61
A3	1.04		3.35		29.51		25.12	87.30
			1					
B1	0.68	2.05	4.43	8.60	3.01	6.29		
B2	3.17	0.73	8.65	2.40	7.06	1.72		
B3	2.30		12.73		8.81			
C	0.81		-		-	-		

15 mins

A1	0.84	1.09	3.37	3.60	13.77	13.58	9.56	8.89
A2	1.78	0.35	4.75	0.61	13.06	0.26	6.53	1.22
A3	0.65		2.67		13.91		10.59	65.50
B1	2.04	2.91	8.76	8.10	10.13	6.48		
B2	3.04	0.47	6.91	0.59	4.89	1.83		
B3	3.64		8.63		4.42			
C	0.55		3.93		10.98		6.50	

30 mins

A1	2.13	0.97	4.05	4.47	10.70	10.81	4.52	5.37
A2	0.38	0.58	6.00	0.79	12.24	0.79	5.86	0.43
A3	0.40		3.37		9.50		5.73	49.6
B1	0.28	1.88	2.75	4.81	3.51	2.56		
B2	2.74	0.80	6.19	1.05	4.16	1.29		
B3	2.61		5.48		0.0			
C	0.60		3.92		10.43		5.91	

60 mins

A1	1.55	1.00	2.32	6.41	64.5	42.92	60.63	35.51
A2	0.71	0.28	4.29	3.15	12.84	15.51	7.84	15.29
A3	0.74		12.61		51.41		38.60	82.7
B1	2.83	2.79	3.27	5.67	2.91	5.07		
B2	2.90	0.08	7.42	1.24	6.95	1.18		
B3	2.64		6.32		5.36			
C	0		2.31		10.84		8.53	

90 mins

A1	1.31	1.10	2.60	4.24	11.76	11.15	7.85	5.81
A2	1.34	0.23	7.35	1.55	10.89	1.73	2.20	1.81
A3	0.64		2.77		10.80		7.39	52.10
B1	2.86	2.99	1.57	1.67	5.86	5.16		
B2	3.68	0.73	1.85	0.09	4.75	1.73		
B3	2.42		1.60		4.88			
C	0.56		4.16		10.19		5.38	

Table : DON and DIN data for the experiment of 21-3-86 (disc. coll) (in umol N.dm-3)

t-set	[Ammonium]	[Nitrate]	[Total N]	[DON]
1-5	1.43	11.05	16.38	3.63
1-10	1.65	10.85	16.30	3.59
1-15	2.35	10.55	80.05	68.10
2-5	1.86	10.89	16.25	3.40
2-10	1.33	10.69	78.59	66.74
2-15	1.00	10.57	61.62	49.68
3-5	0.75	10.92	23.64	11.97
3-10	0.86	10.79	66.19	54.54
3-15	0.91	10.58	53.34	41.84
4-5	0.95	10.97	22.22	10.30
4-10	0.90	10.78	51.15	39.47
4-15	0.75	10.57	21.95	10.63
5-5	0.54	11.28	17.69	5.87
5-10	0.36	10.89	15.15	3.90
5-15	0.51	10.71	14.18	2.96
6-5	0.63	10.91	7.95	3.59
6-10	0.57	10.45	14.80	3.78
6-15	0.55	10.51	59.66	48.60
7-5	0.71	10.73	14.83	3.39
7-10	0.59	10.65	16.86	5.62
7-15	0.62	10.58	15.89	4.69
B1	0.76	11.35	15.81	3.70
B2	0.58	11.33	16.98	5.07
B3	0.64	11.36	17.42	5.42

Table : DIN and DON and Phosphate data for experiment of 20-8-86 [in umol N(P).dm^{-3}]

t(mins)	[Ammonium]	[Nitrate]	[Total N]	PO4	DON
0	0.33	10.7	17.10	0.75	6.89
5	0.46	10.73	15.26	0.73	4.07
10	0.14	10.52	15.50	0.72	4.84
15	0.78	10.61	17.22	0.69	5.83
30	0.24	10.44	16.60	0.63	5.92
60	0.37	9.98	16.00	0.67	5.65
90	0.41	9.74	16.48	0.41	6.22
120	0.51	9.11	16.50	0.36	6.88
180	0.35	8.58	14.52	0.20	5.59
260	0.59	8.00	11.32	0.19	2.73

Values of Nitrate, Ammonium and Phosphate analysis of experiment 7 (8-8-86)

	Nitrate enriched			Ammonium enriched		
	NO_3	NH_4	PO_4	NO_3	NH_4	PO_4
0	44,78	2,22	1,51	13,32	29,43	1,63
5	40,14	1,48	1,86	12,96	21,76	1,33
10	40,48	1,29	1,20	12,66	18,61	0,78
15	40,72	1,39	0,82	12,69	12,62	0,77
30	38,40	1,42	1,12	12,15	6,80	0,71
60	38,16	1,90	0,88	11,48	6,02	0,54
90	37,12	2,16	0,78	10,80	4,41	0,51
120	35,14	3,92	1,32	10,40	2,95	0,41
180	33,52	3,76	1,04	9,45	2,11	0,30

Values for Nitrate, Ammonium and Phosphate of experiment 8 (20-8-86)

	NO_3	NH_4	PO_4	TN	DON
0	10,70	0,33	0,75	17,10	6,86
5	10,73	0,46	0,73	15,26	4,07
10	10,52	0,14	0,72	15,50	4,84
15	10,61	0,78	0,69	17,22	5,83
30	10,44	0,24	0,63	16,60	5,92
60	9,98	0,37	0,67	16,00	5,65
90	9,74	0,52	0,41	16,48	6,22
120	9,11	0,51	0,36	16,50	6,88
180	8,58	0,35	0,20	14,52	5,59
260	8,00	0,59	0,19	11,32	2,73

Appendix 3

Additional Figures

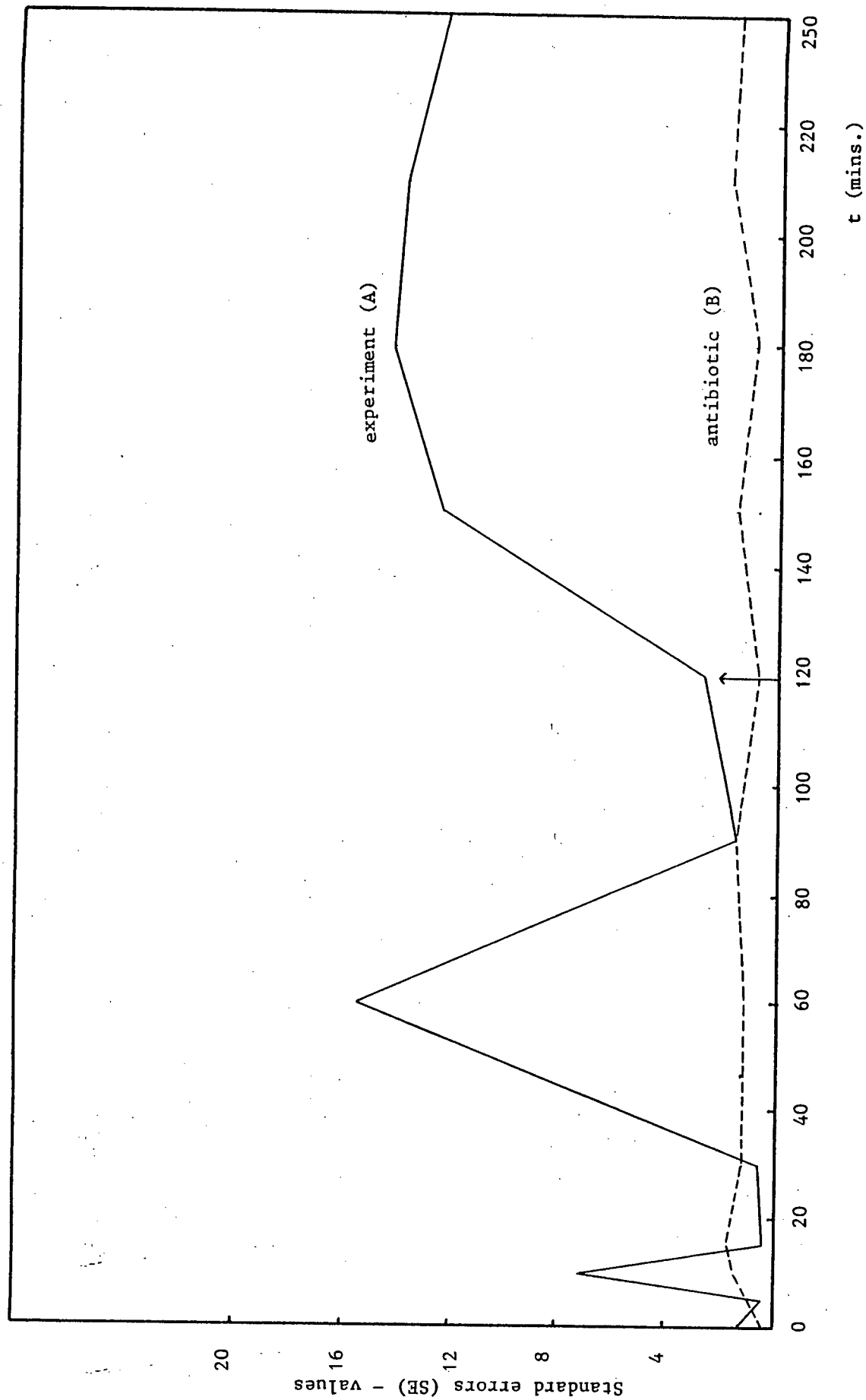

Fig. 6 : Exp. 3 ; Standard error plot (arrow = kelp taken out)

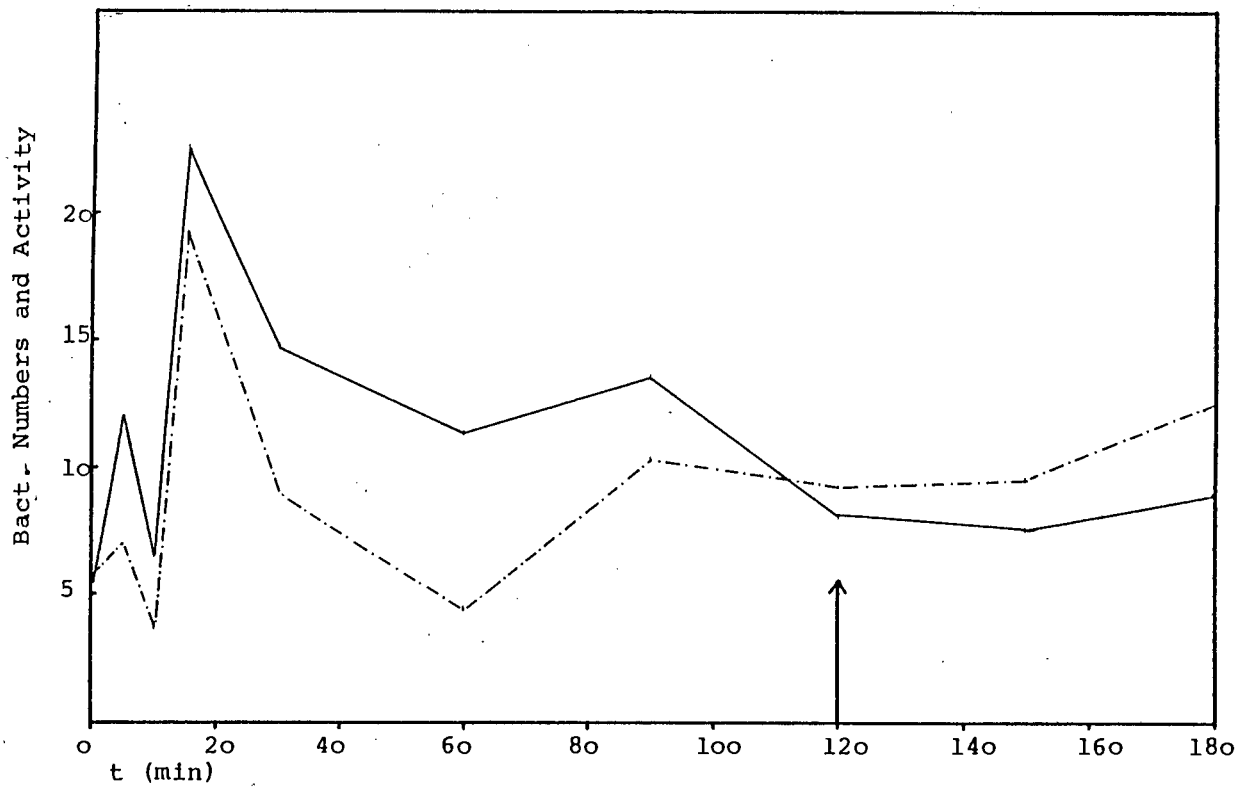

Fig. 12a : Bact. No (Biomass) + Activity of A1 (pg. 63)

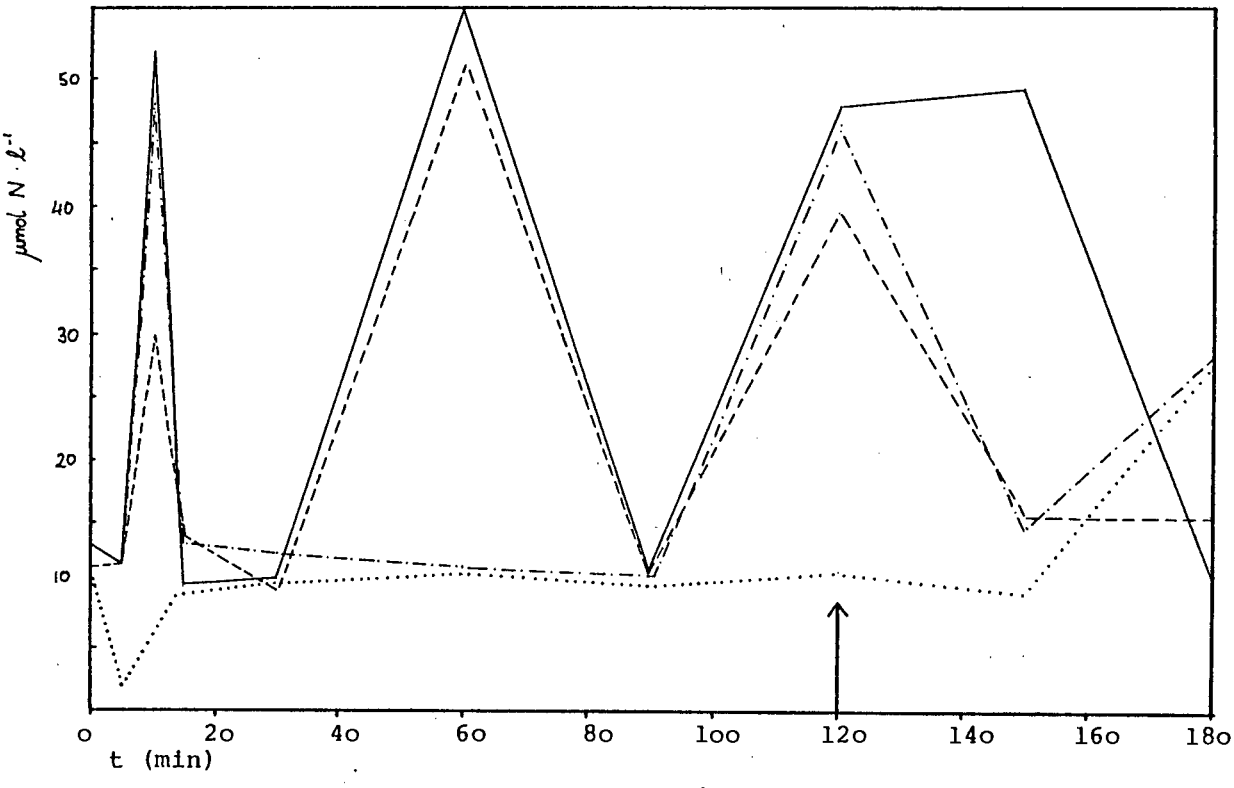

Fig. 4 : DON of A1, A2, A3 + C (pg. 43)

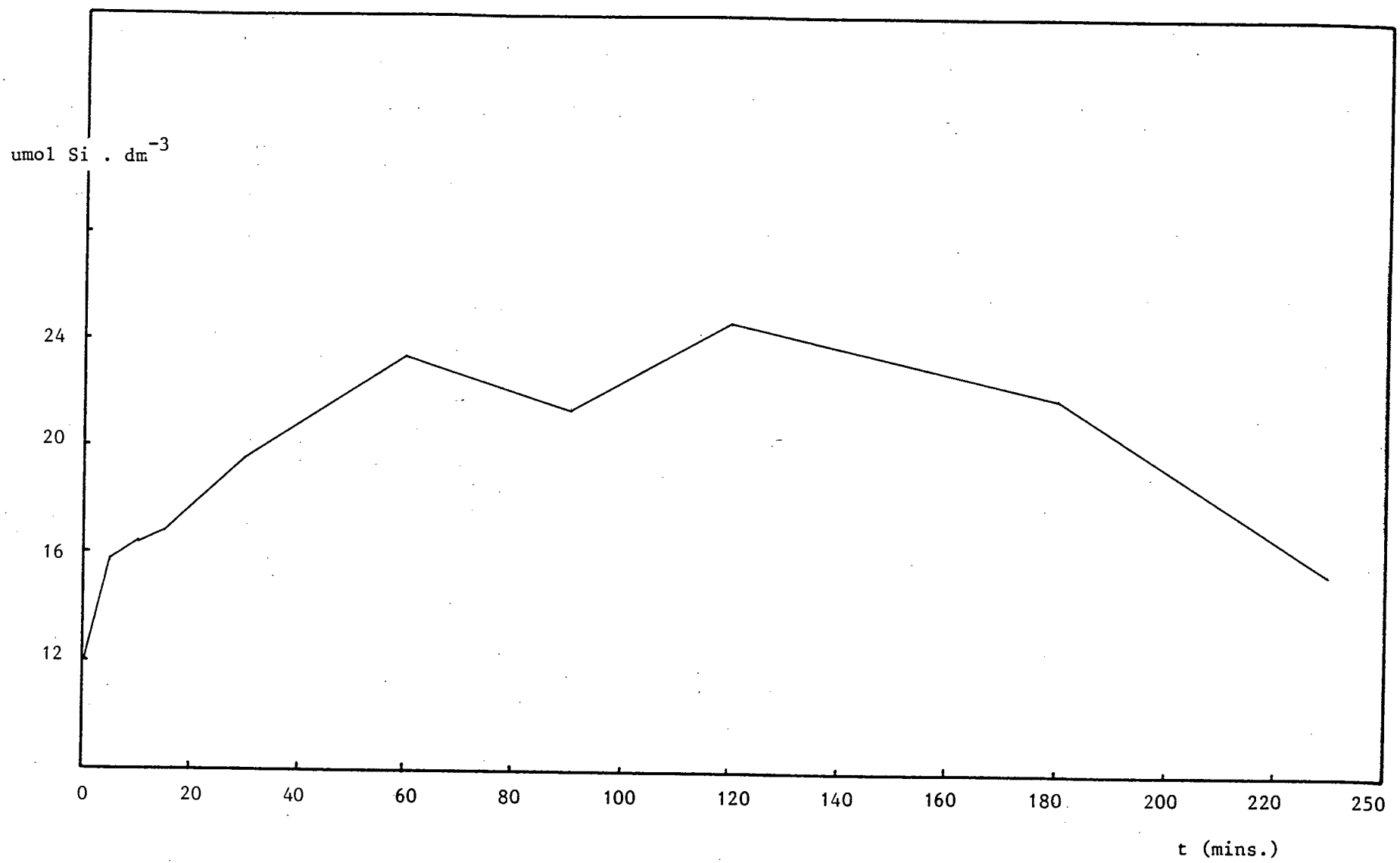

Fig. 25 : Exp. 6 ; Monitoring of silicate levels

NH_4^+

The regression approximates $y = ae^{bx}$ or $y = bx + a$
Using c) :

$$\int_{3o}^{o} (bx + a)\, dx = (-o.443/2 \cdot 3o^2) + (71.58 \cdot 3o) - o$$
$$= 1948.o5 \text{ of total area } (o=bx+a)$$
$$= 1948.o5 \text{ of } 5782.95 = 33.69\%$$

Alternatively : (using...d)

$$\int_{3o}^{o} ae^{bx}\, dx = (-1o3.21/o.o27 \cdot e^{(-.o27(3o))}) - (-1o3.21/o.o27 \cdot e^{o}) = 2122.o83$$
$$= 2122.o83 \text{ of total area } (o=ae^{bx})$$
$$= 55.51\%$$
$$\text{approx.} = 44.66\% \text{ of } NH_4^+ \text{ taken up}$$
$$= 3.521\ \mu mol.\ g^{-1}\ (dw)$$

It follows that TAA + TAN assimilated = 3.669 µmol $N.g^{-1}$(dw)
Total N as calculated percentage from amino acid analysis = 2.466 umol $N.g^{-1}$

Therefore : (2.466x1oo / 3.667) = 67.21% of (TAA+TAN) is assimilated into DFAA after 3o minutes.

Appendix 4

Assimilation of nitrogen into amino acids - calculations.

(see section 4.3.5. Table 8 & section 4.4.2.)

Dry weight of kelp used in experiment : 1o3.5 g

TAN in 1o 1 exp. bucket : (1o8.35.1o.14/1ooo) = 15.17 mg

TAA in 1o 1 exp. bucket : (81.6.1o.14/1ooo) = 11.42 mg

Total initial DFAA conc. : 35.95 $\mu mol.g^{-1}(dw)$

Total 3o mins. DFAA conc. : 52.479 $\mu mol.g^{-1}(dw)$

Total initial N of DFAA : 5.66 $\mu mol\ N.l^{-1}(aa)$

Total 3o mins. N of DFAA : 8.126 $\mu mol\ N.l^{-1}(aa)$

Total N assimilated : 2.466 $\mu mol\ N.l^{-1}(aa)$

Calculation of NO_3^- and NH_4^+ uptake :

NO_3^-

The regression takes the form $y = ae^{bx}$, or $y = bx + a$

Integrating for area under curve : (using...b)

$$\int ae^{bx}\,dx = a\int e^{bx}\,dx = a/b \cdot e^{bx}$$

$$\text{therefore } \int_{3o}^{o} ae^{bx}\,dx = -1o6.6/o.ooo584 \cdot e^{(-o.ooo584.3o)} - -1o6.6/o.ooo584 \cdot e^{o}$$

$$= 317o.148 \text{ of } 182534.247 = 1.737\%$$

Alternatively : (using...a)

$$\int(bx + a)\,dx = (\int bx + \int a)\,dx = \int bx\,dx + a\int dx$$

$$= b/2\ x^2 + ax$$

$$\text{therefore } \int_{3o}^{o}(bx + a)\,dx = -o.o593/2 \cdot (3o)^2 + (1o6.56 \cdot 3o)$$

$$= 317o.115 \text{ of total area } (o=bx+a)$$

$$= 317o.115 \text{ of } 287226.9o7 = 1.1o4\%$$

approx. $= 1.421\ \%\ NO_3^-$ taken up in 3o mins.

$= o.148\ \mu mol.\ g^{-1}(dw)$